STUDIES WITH THE ELECTROCARDIOGRAPH ON THE ACTION OF THE VAGUS NERVE ON THE HUMAN HEART.

I. The Effect of Mechanical Stimulation of the Vagus Nerve.*

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Plates 22 to 24.

The extensive investigation of the action of the vagus nerve on the mammalian heart has given many facts, but has left some questions still unsettled. It has been established by animal experimentation that stimulation of the vagus nerve slows or stops the heart, reduces the force of the auricular beat, and decreases the ability of the heart to conduct the wave of contraction from the auricles to the ventricles. To what degree and in what manner vagus stimulation affects the ventricles directly are questions which are not yet definitely answered. The evidence at hand indicates, however, that the nerve acts mainly on the auricles, and controls the ventricles directly to a less degree. It is difficult to say whether vagus stimulation lowers the excitability of the mammalian heart, but the weight of opinion is in favor of the occurrence of such an action.

Largely through inference, this knowledge of the vagus control of the heart has been applied to man. There are, however, a number of observations which make it highly probable that the facts known to be true for other mammals are true also for man at least as far as the slowing action of the vagus is concerned. It has long been known that pressure over the carotid artery on either side of the neck may cause a long pause in the cardiac rhythm. This effect was first described by Czermak,[1] and the phenomenon, now gen-

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[1] Quoted by Quinke.[2]

erally considered as a result of vagus stimulation, has been discussed by Thanhoffer,[2] Quinke,[3] Hering,[4] Wenckebach[5] and others.

In the course of an observation upon one of our patients involving synchronous auscultation of the precordium and palpation of the left carotid artery, a long pause of the heart occurred. This stoppage of the heart beat was attributed to the mechanical stimulation, by pressure, of the vagus nerve in the neck, and led us to investigate the phenomenon further with the aid of the electrocardiograph.

The patient was placed on a bed beside the electrocardiograph and the electrodes attached to the right arm and left foot, the second lead being usually employed. Records were taken synchronously from the brachial artery, the pulse wave being received by the turgosphygmograph described by Koziczkowsky.[6] During the recording of the curve, digital pressure was made over either the right or left carotid artery at about the level of the cricoid cartilage inside the anterior margin of the sterno-cleido-mastoid muscle. The moments of making and removing the pressure are indicated in most of the records.

The disadvantages of the method are its uncertainty and its danger to the patient. The stimulation cannot, of course, be measured accurately quantitatively, or as to its duration, and it is impossible to tell when the actual stimulation begins. Consequently, no attempt has been made to measure the latent period of vagus activity.

That serious symptoms may follow pressure on the vagus nerve in the neck is known, but there are no recorded fatalities. The human heart seems to show the same ready escape from the vagus inhibition that Hough[7] has shown to occur in the lower animals.

[2] Thanhoffer, Die beiderseitige mechanische Reizung des Nv. vagus beim Menschen, *Centralbl. f. d. med. Wissensch.*, 1875, xiii, 403.

[3] H. Quinke, Über Vagus Reizung beim Menschen, *Berl. klin. Wchnschr.*, 1875, xii, 189.

[4] H. E. Hering, Die Functionsprüfung der Herzvagi beim Menschen, *München. med. Wchnschr.*, 1910, lvii, 1931.

[5] K. F. Wenckebach, Effects of Digitalis on the Human Heart, *Brit. Med. Jour.*, 1910, ii, 1600.

[6] Koziczkowsky, Über "Turgosphygmographie" und ihre Verwendung für Pulsdruckbestimmungen, *Berl. klin. Wchnschr.*, 1907, xliv, 369.

[7] Hough, On the Escape of the Heart from Vagus Inhibition, *Jour. Physiol.*, 1895, xviii, 161.

We have observed no unpleasant symptoms in our patients, and in only one case has there been even the slightest evidence of circulatory inadequacy as a result of the procedure. In this instance, a transient dizziness, suggesting cerebral anemia, occurred. It must be borne in mind, however, that stoppage of the heart by vagus stimulation, when the " factors of safety " in the heart are already greatly reduced, may be not without considerable risk. Pressure on both sides of the neck is probably hardly justified in man, although we have produced it with much caution in one case. Quinke[3] considers pressure on one vagus to be not without danger, while Hering[4] reports that it has been carried out in his clinic several hundred times without any ill effects, and advises it as a diagnostic measure.

The method of recording the effects of vagus pressure in man by the electrocardiograph affords a valuable means of comparing the human physiology with that of lower mammals. The method has been applied experimentally in animals, chiefly in dogs, by Einthoven,[8] Hering,[9] Kahn,[10] Kraus and Nicolai,[11] Rothberger and Winterberg,[12, 13] and Samojloff.[14] All these observers stimulated the vagus nerve directly with an electric current. The striking similarity of our results to theirs is a forcible argument in support of the statement that effects on the human heart caused by pressure over the carotid artery are due entirely to vagus stimulation.

Although we have studied rather extensively the effect of vagus pressure on one patient whose heart showed no anatomical abnor-

[8] W. Einthoven, Le télécardiogramme, *Arch. internat. de physiol.*, 1906, iv, 132. W. Einthoven, Weiteres über Elektrokardiogramm, *Arch. f. d. ges. Physiol.*, 1908, cxxii, 517.

[9] Hering, Experimentelle Studien an Säugethieren über das Elektrokardiogramm, *Arch. f. d. ges. Physiol.*, 1909, cxxvii, 155.

[10] Kahn, Beiträge zur Kenntnis des Elektrokardiogrammes, *Arch. f. d. ges. Physiol.*, 1909, cxxvi, 197.

[11] Kraus and Nicolai, Das Elektrokardiogramme, Leipzig, 1910.

[12] Rothberger and Winterberg, Über den Ausdruck der Wirkung der Herznerven im Elektrokardiogramm, *Zentralbl. f. Herz u. Gefässkrankheiten*, 1911, iii, 133.

[13] Rothberger and Winterberg, Über die Beziehungen der Herznerven zur Form des Elektrokardiogramms, *Arch. f. d. ges. Physiol.*, 1910, cxxxv, 506.

[14] Samojloff, Weitere Beiträge zur Elektrophysiologie des Herzens, *Arch. f. d. ges. Physiol.*, 1910, cxxxv, 417.

malities, our investigation has been conducted for the most part on patients showing auricular fibrillation and on children with chronically diseased hearts following rheumatism. The action of atropin and digitalis on the effects produced by pressure has also been studied. Our present communication, however, will deal with the results obtained on hearts uninfluenced by digitalis, and will include only those cases showing no anatomical cardiac abnormalities and those showing auricular fibrillation.

THE EFFECT OF PRESSURE ON THE VAGUS NERVE ON AN ANATOMICALLY NORMAL HEART.

We have made a somewhat extended study of a man, whose heart responded markedly to vagus pressure, and whose physical signs revealed no definite cardiac abnormalities.

He was fifty-seven years old and was admitted to the hospital complaining of attacks of sensations of oppression about the heart, usually coming on at night and lasting ten or fifteen minutes. The attacks had occurred at intervals of every few days during a period of about six months and prevented him from working as a bricklayer. There was no definite pain and no peculiar sensations extended down the left arm. He had smoked excessively, used alcohol rather freely, and worked moderately hard. There was no history of rheumatism, scarlet fever, or syphilis, and the Wassermann reaction was negative.

The patient had a "neurotic" appearance, and the knee jerks were very slightly exaggerated. Otherwise, the physical examination revealed no anatomical abnormalities, except a slight increase in cardiac dullness. The dullness extended 5 cm. to the right of the midsternal line in the fourth space and 15 cm. to the left in the fifth space. The heart rate was 54 per minute; the sounds somewhat distant and clear, although the second aortic sound was high pitched and ringing in quality. The presence of albumin and many hyaline casts in the urine led to a diagnosis of chronic interstitial nephritis. The blood pressure at the time of admission, with the patient in a recumbent position, was systolic 157 mm. Hg., and diastolic 83 mm. Hg. Numerous observations for two weeks after admission showed an unstable blood pressure. With the patient at rest in bed, the systolic pressure varied from 150 to 120 mm. Hg. It was, for the most part, about 125 systolic and 75 mm. Hg. diastolic by the auscultatory method. After exercise, blood pressure and pulse rate estimations showed that the heart responded to the extra work by a somewhat abnormal increase in rate, rather than by an increase in output of blood per minute.

The patient was seen in several of the attacks of sensations of oppression, at which times the heart was markedly irregular. The face became flushed, but there was no pain or dyspnoea. The arhythmia had always ceased before graphic or electrocardiographic

records could be made. It seemed, however, to consist of extrasystoles, following each other rapidly and in groups. Definite ventricular extrasystoles occurred towards the end of the attacks when the heart was approaching its normal rate and rhythm.

The effect of vagus pressure was studied by means of electrocardiograms on several occasions. The second lead, the right arm and left foot, was always used, and tracings from the brachial artery were recorded simultaneously. The effects of pressure on the right and on the left side of the neck were studied. Records were made in the same manner after atropin had been administered. The patient was then put under the influence of digitalis and the effects of this drug on the results of vagus pressure were observed. The observation with digitalis will not be considered at this time, but will form the subject of a later communication. The curves reproduced show the electrocardiogram without vagus pressure (figure 1), the effect of pressure on the right side of the neck (figure 2), the effect of pressure on the left side of the neck (figure 3), and the effect of pressure over the right vagus nerve after atropin had been administered (figure 4). The normal electrocardiogram (figure 1) is shown only for comparison with the others.

The records obtained after the administration of atropin demonstrate conclusively that we are dealing with effects of vagus stimulation. It is a well known fact, established both experimentally and in man, that the drug in sufficient doses blocks the effect of vagus stimulation on the heart. The record (figure 4) was made after 1.2 milligram (1/50 gram) of atropin sulphate had been administered hypodermically in two doses forty-five minutes apart. A half hour elapsed between the second dose and the taking of the record. It is seen that except for a slight slowing, occurring several beats after the pressure was exerted, no effects can be detected. This was the only occasion in numerous attempts when marked effects of pressure were not readily produced.

All the records from the patient have been analyzed to determine what effect the mechanical stimulation of the vagus nerve exerted on the various cardiac properties,—rhythmicity, conductivity, contractility, and excitability,—which have been shown experimentally to be under vagus control.

In order to determine changes in rhythm (or rate), the time between the recurrence of a corresponding point in the electrical curves has been measured. The beginning of the ventricular complex, which is well defined in all the curves as the onset of the negative Q wave, has been chosen. Therefore the Q–Q time represents the length of each ventricular cycle. Changes in conductivity are determined by measuring the time between the first indication of auricular stimulation, the footpoint of the P wave, and the onset of the Q wave.

There has always been considerable difficulty in demonstrating conclusively changes in contractility of the ventricle due to vagus stimulation, except when suspension curves are possible, and as far as we know no previous attempt has been made to do this in man. Hering[4] has referred to the difficulties, however. We believe nevertheless that some evidence on the question may be obtained by measuring the time elapsing between the stimulation of the ventricles and the appearance of the brachial pulse.

We have called this interval of time the Q-B time, as it is measured from the first indication of the Q wave and the footpoint of the brachial pulse curve. This time is composed of three factors, (1) the interval between stimulation and contraction of the ventricle, (2) the time required by the ventricle to raise the intraventricular pressure above the intra-aortic pressure and so open the aortic valves (the presphygmic period), and (3) the time of pulse wave transmission from the aortic valves to the brachial artery. In our previous work[15] on the presphygmic period, we have shown that the transmission time, at least to the carotid artery, is very short and fairly constant, so that variations are practically within the limit of error of the method of measuring the transmission time; therefore, the variations in transmission time to the brachial are probably insignificant. We have as yet incomplete and unpublished evidence that the onset of ventricular stimulation has a constant relation to the onset of ventricular contraction. It seems probable, therefore, that the major part of any variations which occur in the Q-B time are due to variations in the length of time required for the left ventricle to overcome the intra-aortic pressure. This time in turn may be considered as an index to the strength of contraction of the left ventricle, provided the intra-aortic pressure is constant. Changes in this last factor, as well as in the length of the preceding diastole, must also be taken into account, however, as we have previously shown, in attempting to draw conclusions from variations in the presphygmic period. These points will be considered when the individual curves are discussed.

[15] Robinson and Draper, A Study of the Presphygmic Period of the Heart, *Arch. Int. Med.*, 1910, v, 168.

The length of each systole has been included in the analysis, in order to see if variations are present which may throw light on the question of changes in contractility. It has been measured by determining the time elapsing between the first evidence of ventricular stimulation and the end of the T wave.

Finally the heights of the P wave and of the R wave have been measured from points designated on the curves, from the bottom of the rather broad line forming the curve to the inside peak of the P wave and to the crest of the R wave.

All of these data from figures 1, 2, 3 and 4 are tabulated as follows:—

FROM FIGURE 1.*

Curve 16. No Vagus Stimulation.

Number of cycle.	Q–Q.	Systole.	Preceding diastole.	Q–B.	P–Q.	Height of	
						P.	R.
1	0.92	0.355	——	0.170	0.137	—	——
2	0.92	0.36	0.565	0.178	0.144	1.4	13.0
3	0.96	0.365	0.56	0.172	0.136	1.4	12.7
4	——	0.36	0.595	0.174	0.137	1.4	12.3

* Time measured in seconds, heights in millimeters.

FROM FIGURE 2.

Curve 16.2. Right Vagus Pressure.

Number of cycle.	Q–Q.	Systole.	Preceding diastole.	Q–B.	P–Q.	Height of	
						P.	R.
1	0.91	0.37	——	0.177	——	—	——
2	0.94	0.36	0.54	0.168	0.144	1.5	12.5
*3	0.93	——	0.58	0.172	0.144	1.5	12.5
†4	3.77	0.38	——	0.173	0.140	1.5	12.3
5	1.34	0.40	3.39	0.170	0.155	1.5	13.5
6	1.18	0.39	0.94	0.173	0.164	1.4	13.0
7	1.06	0.38	0.79	0.178	0.144	1.3	12.7
8	1.03	0.38	0.68	0.172	0.144	1.5	12.6
9	——	0.39	0.65	0.167	0.144	1.6	12.6

* Stimulus applied soon after third systole.

† Pressure in the neck 4 seconds. Removed shortly before beginning of fifth cycle.

FROM FIGURE 3.

Curve 20.3. Left Vagus Pressure.

Number of cycle.	Q–Q.	Systole.	Preceding diastole.	Q–B.	P–Q.	Height of	
						P.	R.
1	0.84	0.36	——	0.186	0.135	2.3	16.6
2	0.83	0.36	0.48	0.186	0.131	1.8	16.6
*3	0.86	0.34	0.47	0.179	0.137	1.8	16.6
4	0.99	0.35	0.52	0.170	0.150	1.8	16.5
5	1.30	0.36	0.65	0.174	0.150	1.9	15.6
6	1.15	0.36	0.95	0.170	0.183	2.0	16.3
7	1.12	0.36	0.79	0.169	0.162	2.0	16.1
8	1.07	0.36	0.76	0.169	0.153	1.8	16.1
9	1.06	0.35	0.71	0.177	0.141	2.0	16.6
10	——	——	0.71	——	0.151	2.0	16.5

* Vagus pressure here. No appreciable change in T wave.

FROM FIGURE 4.

Curve 17. Right Vagus Pressure. After 1.2 mg. Atropin.

Number of cycle.	Q–Q.	Systole.	Preceding diastole.	Q–B.	P–Q.	Height of	
						P.	R.
1	0.64	0.30	——	0.184	0.122	—	——
2	0.61	0.29	0.34	——	0.122	1.4	10.8
3	0.56	0.30	0.32	0.200	0.130	1.5	10.8
*4	0.57	——	——	——	——	—	——
5	0.63	0.315	——	0.200	0.130	1.6	11.1
6	0.66	0.31	0.315	0.180	0.117	1.5	11.4
7	0.73	0.32	0.35	0.183	0.117	1.5	11.1
8	0.75	0.32	0.41	0.183	0.132	1.5	10.8
9	0.79	0.32	0.43	0.186	0.121	1.4	11.0
10	0.82	0.32	0.47	0.202	0.132	1.0	10.8
†11	0.80	0.32	0.50	0.198	0.140	1.5	11.0

* Vagus pressure here. † Vagus pressure released.

It is seen in figure 2 that pressure over the right vagus nerve, lasting four seconds, produced an immediate, marked slowing or pause in the cardiac rhythm, both the auricle and ventricle being affected. We are dealing therefore with an inhibitory action of the vagus on rhythmicity, which occurs very soon after vagus stimulation, and which gradually disappears. The conductivity is lowered as the P–Q time increases from about 0.144 second to 0.155 second and then to 0.164 second. After two heart beats the time returns to that seen before vagus pressure.

The Q–B time is seen to remain practically unchanged, as our method of measuring time relations does not approach accuracy

closer than 0.01 second. These Q–B figures indicate, therefore, that the left ventricle requires about the same period of time to overcome the intra-aortic pressure after a very long diastolic pause as after a short one, in spite of the facts that the ventricle has had a much longer time in which to store up energy-producing material, and that the intra-aortic pressure has probably fallen below that maintained with the usual heart rate. The low level to which the turgographic record from the brachial artery falls is an indication of a lowered intra-arterial diastolic pressure. It is also seen that the height of the brachial waves following the long pauses is not so great as the waves occurring later in the curves.

The length of systole, as measured in the electrocardiograms, is somewhat increased after vagus stimulation, but this may be only a mechanical result due to a slight overfilling of the ventricles during the long diastoles. In a record in which right vagus pressure produced less marked slowing, this lengthening of systole does not occur. Variations in the length of systole allow, therefore, no conclusions as to variations in the force of ventricular contractions. We have, however, in the other points considered, at least presumptive evidence in this case that vagus stimulation causes a diminution in the force of ventricular contractions. Much more definite evidence of this effect has been obtained, in practically all the cases which we have studied, by a marked weakening of the heart sounds or of mitral systolic murmurs when present, by a diminution of the force of the radial pulse, and in some instances by a diminution of the size of the graphic record of the apex beat taken from the chest wall. The curve affords no evidence as to any changes in excitability of auricles or ventricles which may have occurred.

No significant changes in the size or form of the P wave can be made out in the electrical curve, while there is a definite increase in the height of the R wave following right vagus pressure. The meaning of this increase in the action current accompanying ventricular systole is not clear. Rothberger and Winterberg[13] have noted a decidedly increased height of the R wave in the electrocardiograms from dogs after vagus stimulation, and they believe that it is a purely inhibitory effect, perhaps an expression of a slowed cardiac rhythm, as a decrease in the size of the wave was

obtained by stimulation of accelerator nerves. The fact that the increased R waves follow vagus stimulation makes it appear unlikely that they represent increased ventricular power. The work of Hering,[16] who showed that the weaker beat of a heart in which alternation had been produced was accompanied by a larger R wave than the stronger beat, also leads to the conclusion that the increased R wave has some other significance than an increase in ventricular power. Slight elongation and flattening of the T wave is seen after the stimulation of the nerve. The significance of this change, which has also been observed, is not clear.

The record obtained with left vagus pressure (figure 3) shows a much less marked slowing, the longest Q–Q interval (representing the cardiac cycle) being 1.30 seconds as compared to 3.77 seconds resulting from right vagus pressure. The fact that right vagus pressure produces greater slowing than pressure on the left side was seen in practically all cases where pressure was made on each side. There is, moreover, a difference in the method of response from pressure on the two sides of the neck. When response occurred from pressure on the right vagus, the maximal lengthening of the cadiac cycle was seen at once. With left-sided pressure, however, in both the curves which we obtained, the first effect was the production of a submaximal lengthening, while the second cardiac cycle showed the maximal slowing, and several succeeding cycles were longer than the one in which the effect of vagus stimulation was first seen.

In spite of a lessened degree of slowing, left vagus stimulation has produced a distinctly more marked delay in conduction than pressure on the right side of the neck. The records obtained with left vagus pressure, especially an unpublished curve, show a noticeable decrease in the Q–B time in response to the lengthening of the preceding diastole. Thus in the unpublished curve the Q–B time is 0.177 second when the preceding diastole is 0.445, while it shortens to 0.152 second when the preceding diastole is 0.9 second in duration. This would make it appear probable that the left vagus stimulation had produced very little reduction in the force of ventricular systole.

[16] Hering, Über den Pulsus irregularis perpetuus, *Deutsch. Arch. f. klin. Med.*, 1908, xciv, 185.

The height of the P wave shows no significant changes, while in both the published and the unpublished records the R wave shows a diminution following the long diastoles, an opposite effect to that obtained by right vagus stimulation.

On account of our somewhat uncertain manner of stimulating the vagus, no positive conclusions of a comparative nature can be drawn. We are justified, however, in saying that it is highly probable that the two vagi influence the various cardiac properties to a somewhat different degree,—at least when stimulated mechanically by pressure from without.

The various experimenters, who have obtained electrocardiograms from dogs during vagus stimulation, have shown that the heart is markedly slowed, that the conduction time is increased, and that the auricular contractions are sometimes blocked. The P wave is usually decreased in size, while the R wave is increased; a decrease or flattening of the T wave has also been noted. The curves obtained by means of the electrocardiograph from animals in which the vagus nerve is stimulated electrically, and those obtained from our patient in whom the vagus was stimulated by pressure, show a close resemblance, and afford striking evidence that the vagus nerve affects the human heart in a way which is very similar to its effect on the heart of lower mammals.

SUMMARY.

Our records show that stimulation of the vagus nerve by pressure in a man with an anatomically normal heart has produced a marked slowing of both auricles and ventricles, a depression of conductivity of the heart beat from auricles to ventricles, and probably has resulted in a diminution in the force of contraction of the left ventricle.

Stimulation of the right vagus nerve has apparently in our case a more marked effect on the rate of the heart and on the force of ventricular contraction, than has stimulation of the left. The left vagus stimulation seems to have a more marked effect on the conduction of the cardiac stimulus from auricles to ventricles. While no significant changes have been noted in this case in the action current due to auricular contraction, right-sided vagus stimulation

seems to increase the action current of the ventricles, giving rise to an increased R wave, while left vagus stimulation apparently produces the opposite effect. There is no evidence in our curves that any changes in excitability of the auricles or ventricles are produced by vagus stimulation. Our records supply definite evidence to the effect that the vagus nerve controls the heart in a way which is very similar in man and in other mammals.

THE EFFECT OF VAGUS STIMULATION ON HEARTS SHOWING AURICULAR FIBRILLATION.

Many of the patients with auricular fibrillation and constantly irregular ventricular action, which we have studied, have shown marked response to pressure on the vagus nerve. Figures 5, 6 and 7 are from three cases in which vagus pressure produced the longest pauses in the cardiac rhythm. Two of the patients were men over sixty, while the third was a woman of forty-four years. In all, evidence of cardiac disease had been present for a number of years and the hearts of all showed enlargement on percussion. The patient from whom figure 6 was obtained showed signs of great enlargement. Venous tracings always showed curves typical of auricular fibrillation or *pulsus irregularis perpetuus*. The A wave was always absent, the C and V waves were prominent, and often fine undulations could be seen in the curves during diastole. These small waves were of about the same rate as the undulations in the diastolic periods in the electrocardiograms, and they doubtless represent the same phenomenon. The work of Rothberger and Winterberg[17] and of Lewis[18] leaves no reasonable doubt that this phenomenon is auricular fibrillation. In all the cases pressure was made on the right side of the neck. The results of an analysis of figures 5, 6 and 7 are as follows:—

[17] Rothberger and Winterberg, Vorhofflimmern und Arhythmia perpetua, *Wien. klin. Wchnschr.*, 1909, xxii, 839.

[18] Lewis, Auricular Fibrillation and its Relationship to Clinical Irregularity of the Heart, *Heart*, 1910, i, 306.

From Figure 5.

Curve 1.4.

Number of cycle.	R-R.	Systole.	Preceding diastole.	R-B.	Fibrillation rate.
1	——	0.337	——	0.200	——
2	0.959	0.466	0.622	0.206	300
*3	1.350	0.387	0.884	0.202	375
4	3.710	0.402	3.323	0.213	——
5	0.518	0.402	0.116	0.282	480
6	2.200	0.373	1.798	0.196	——
7	1.094	0.336	0.721	0.211	——
8	0.875	0.339	0.539	0.205	——
9	0.708	——	0.369	0.200	——

From Figure 6.

Curve 15.3. Third Lead.

Number of cycle.	R-R.	Systole.	Preceding diastole.	R-B.	Height of R.
1	——			——	15 negative.
2	0.564	0.305	0.259	0.223	15 "
3	0.574	0.317	0.257	0.243	15 "
*4	0.737	——	——	0.192	14.5 "
5	0.775	0.326	0.449	0.229	15 "
6	1.437	0.352	1.185	0.175	15 "
7	0.425	0.320	0.105	Frustrated.	11 "
8	1.120	0.365	0.755	0.175	15.25 "
9	6.550	0.365	6.185	0.189	11 "
10	0.442	0.343	0.099	Frustrated.	26.50 positive.
11	1.650	0.412	1.238	0.188	15 negative.
12	0.765	0.337	0.428	0.290	16.40 "
13	0.622	0.343	0.279	0.237	15.60 "
14	0.960	0.333	0.627	0.199	16.70 "
15	0.475	0.333	0.142	Frustrated.	12.25 positive.
16	0.925	0.370	0.550	0.190	15 negative.
17	0.525	——	——	Frustrated.	11.90 positive.
18	1.585	——	——	0.185	15.50 "

From Figure 7.

Curve 3.1.

Number of cycle.	R-R.	Systole.	Preceding diastole.	R-B.	R.	Fibrillation rate.
1	——	0.319	——	0.200	14.00	——
2	1.240	0.344	0.921	0.170	13.50	300
3	0.860	0.362	0.516	0.187	12.50	——
*4	1.307	0.410	0.945	0.182	13.50	——
5	1.095	0.347	0.685	0.197	15.00	375
6	2.612	——	2.226	0.195	16.00	420

* Vagus stimulation.

As the R wave represents the first evidence of ventricular activity, this letter is used instead of Q as above.

The most striking feature in these cases is that vagus stimulation causes long delays in the ventricular rhythm, but is without any definite effect on the auricular fibrillations. At first sight it would appear that we have here evidence that the vagus nerve acts directly on the ventricle. When the pathological physiology of these cases is considered, however, this marked disturbance of ventricular rhythm will be seen to depend probably on a reduction in the conductivity of the heart. Cushny[19] has pointed out that "during auricular fibrillation, impulses are probably showered upon the auriculoventricular fibers" at a rapid rate and only a certain proportion are conveyed to the ventricles. The ventricles are stimulated at irregular intervals, but the number of effectual stimuli per minute depends upon the conductivity of the auriculoventricular fibers.

As one of the most obvious effects of vagus stimulation is lowering of conductivity, it seems clear that pressure over the right vagus in these cases has caused a complete blockage of stimuli from the fibrillating auricles, lasting in one case as long as 6.55 seconds. We have no evidence as to whether this striking effect depends upon an increased susceptibility of the conducting fibers to vagus stimulation or whether it depends on the stimuli from the fibrillating auricles being of such a nature that only slight lowering of the conductivity succeeds in blocking them. Vagus stimulation may produce also some quantitative change in the auricular activity which is not to be detected in the electrical curves and which yet makes it incapable of sending stimuli to the ventricles. It may be said, however, that many stimuli are probably being blocked constantly in this form of heart lesion, and this heightened ability to block stimuli from the fibrillating auricles probably represents a conservative phenomenon. Digitalis, which is well known to possess the power of lowering conductivity, acts with especial benefit in cases showing auricular fibrillation, slowing the ventricles, as Cushny[19] suggests, probably by blocking a larger proportion of stimuli than would otherwise be the case. This relation of digitalis to vagus action, which has been suggested by Wenckebach, will be discussed more fully in a later communication.

[19] Cushny, Irregularity of the Heart and Auricular Fibrillation, *Am. Jour. of Med. Sciences*, 1911, cxli, 826.

A number of records from the three cases here reported and from others have been analyzed in order to determine whether vagus stimulation has produced changes in the electrocardiographic records of auricular fibrillation. In figure 7, where the fibrillations produce fairly distinct waves, a slight increase in rate occurs; although no conclusions are warranted from this observation, it does agree with some experimental findings (Cushny). Moreover it indicates that vagus stimulation does not prevent or lower the rate of auricular fibrillation as seen in diseased human hearts.

The effect of vagus stimulation on the fibrillating auricles in animals is an unsettled question, as various results have been reported. Thus Fischel,[20] McWilliam,[21] Philips[22] and Cushny[23] have shown that vagus stimulation may restore the rhythm of the auricle after it has been thrown into fibrillation by direct electrical stimulation. Kronecker and Spallitta[24] found that stimulation of the vagus does not abolish auricular fibrillation, and Rothberger and Winterberg[25] have advanced strong evidence, in their electrocardiographic curves, to the effect that the apparent arrest of fibrillation is not complete, but is really the expression of a powerful depression of the force of auricular contraction. Several observers have shown that stimulation of the vagus nerve alone may throw the auricles into fibrillation, and Winterberg[26] found that weaker stimulation of the auricles caused fibrillation more readily during vagus stimulation than when the nerve was not stimulated. Garrey,[27] who has

[20] Fischel, Über Tonusänderungen und die anderen graphisch an den vier Abtheilungen des Säugethierherzens bei elektrischer Reizung desselben zu ermittelnden Erscheinungen, *Arch. f. exp. Path. u. Pharmakol.*, 1897, xxxviii, 228.

[21] McWilliam, Fibrillar Contraction of the Heart, *Jour. Phys.*, 1887, viii, 296.

[22] Philips, Les trémulations fibrillaires des oreillettes et des ventricules du coeur de chien, *Arch. internat. de physiol.*, 1905, ii, 271.

[23] Cushny, The Therapeutics of Digitalis and its Allies, *Am. Jour. Med. Sc.*, 1911, cxli, 469.

[24] Kronecker and Spallitta, La conduction de l'inhibition à travers le coeur du chien, *Arch. internat. de physiol.*, 1905, ii, 223.

[25] Rothberger and Winterberg, Über das Elektrokardiogramm bei Flimmern der Vorhöfe, *Arch. f. d. ges. Physiol.*, 1910, cxxxi, 387.

[26] Winterberg, Studien über Herzflimmern. 1st part, *Arch. f. d. ges. Physiol.*, 1907, cxvii, 223.

[27] Garrey, Some Effects of Cardiac Nerves upon Ventricular Fibrillation, *Am. Jour. Physiol.*, 1908, xxi, 283.

recently experimented on the subject and has reviewed the evidence, considers that the fact is established in animals that auricular fibrillation, at least in some stages, can be controlled by the vagi.

In comparing the results of animal experiments with our observations on the human heart, it must be remembered that the conditions are doubtless very different. What may be spoken of as fresh fibrillation produced in normal auricles and dealt with immediately, is probably a very labile condition from which the auricles tend to revert to rhythmicity. But the heart of a patient, who has had a constantly irregular pulse over a long period, presents an auricle with old established fibrillation which has shown no tendency, perhaps for years, to beat coordinately. In these cases vagus stimulation has apparently marked effect on conductivity, while it does not seem to affect auricular activity. Consequently, there may be some inherent difference in the condition of the auricle itself which renders its response to vagus stimulation nil. Our observations throw no light on the nature of this suspected difference.

The effect of right vagus pressure on the force of ventricular systole is apparent. In figure 5 for instance the brachial pulse wave is much reduced in size, and the time, in spite of the long preceding diastole, between the ventricular stimulus and the appearance of the brachial pulse wave is increased. Figure 7 affords another example of this condition. Auscultation over the heart in these cases revealed a great diminution in the force of the heart sounds and left no doubt in our minds that following vagus stimulation there was a distinct decrease in the force of ventricular contractions. There seems no other interpretation of this phenomenon than to consider that in hearts with auricular fibrillation, the right vagus nerve acts directly on the ventricles, causing a marked diminution in the force of contraction.

The effect on the height of the R wave is apparently variable: in curve 7 there is an increase in height, no effect is apparent in curve 6, while in curve 5 the negative wave, representing the ventricular contraction occurring after the long pause, is much reduced in size, although its form is unchanged.

In figures 5 and 6 abnormal ventricular complexes are seen in the electrical curves following the long inhibitory pauses. We have

seen them in several other curves. Kahn[10] and Hering[9] have observed that abnormal complexes occur during or after vagus stimulation in dogs, and Hering considers that they may be due to ventricular systoles which are greatly weakened by vagus action. Without discussing Hering's arguments, it must be said that these abnormal ventricular complexes seem to us to represent ventricular systoles arising from some abnormal point in the ventricle itself. The significance of the abnormal curves has been discussed by James and Williams,[28] Winterberg[29] and others. The presence of the ectopic ventricular beats following periods of long ventricular quiescence suggests that in the absence of stimuli from the auricles, some abnormal point is attempting to carry on the function of stimulus formation; thus affording another example of a conservative process similar to that seen in some cases of complete heart-block. In the case from which tracing 6 was obtained, ectopic ventricular beats occur frequently, independent of vagus stimulation. The absence of such beats during the long pause of the ventricle suggests the possibility of a lowering of excitability of the ventricle by vagus action. This indefinite evidence is all that our records give in regard to the effect of the vagus stimulation in these abnormal hearts. Roy and Adami[30] believed that their experiments show that direct excitation of the vagus may so lower the excitability of the ventricles that the contractions may not extend over the whole of the walls. If such a result is possible, the abnormal ventricular complexes in our figures may be explained as representing these partial ventricular contractions.

SUMMARY.

In hearts showing auricular fibrillation mechanical stimulation of the right vagus nerve causes, as a rule, marked slowing or stoppage of ventricular rhythm, without producing any appreciable effect in the electrocardiographic record of the auricular fibrillation. The

[28] James and Williams, The Electrocardiogram in Clinical Medicine; II, The Electrocardiogram in some Familiar Diseases of the Heart, *Am. Jour. Med. Sc.*, 1910, cxl, 644.

[29] Winterberg, Das Elektrokardiogramm, seine theoretische und praktische Bedeutung, *Med. Klinik*, 1911, vii, 761 and 804.

[30] Roy and Adami, Contribution to the Physiology and Pathology of the Mammalian Heart, *Brit. Med. Jour.*, 1892, i, 428.

ventricular pauses are apparently due to the blocking of stimuli from the auricles. The force of ventricular systole is distinctly weakened for several beats after vagus stimulation, and ectopic ventricular systoles have been seen in several instances, apparently the result of the vagus action. There may, in some cases, be lowered excitability of the ventricles, while no constant change is seen in the size of the electrical complexes representing ventricular systole.

EXPLANATION OF PLATES.

PLATE 22.

FIG. 1. Curve from patient with an anatomically normal heart. Patient No. 1.

FIG. 2. The effect of pressure on the right vagus nerve. Patient No. 1.

FIG. 3. The effect of pressure on the left vagus nerve. Patient No. 1.

PLATE 23.

FIG. 4. The effect of pressure on the right vagus nerve after the administration of atropin. Patient No. 1.

FIG. 5. The effect of pressure on the right vagus nerve on a heart with auricular fibrillation. Patient No. 2.

PLATE 24.

FIG. 6. The effect of right vagus pressure on a heart with auricular fibrillation. Patient No. 3. The electrocardiogram was taken from the third lead, *i. e.*, left arm and left foot.

FIG. 7. The effect of right vagus pressure on a heart with auricular fibrillation. Patient No. 4.

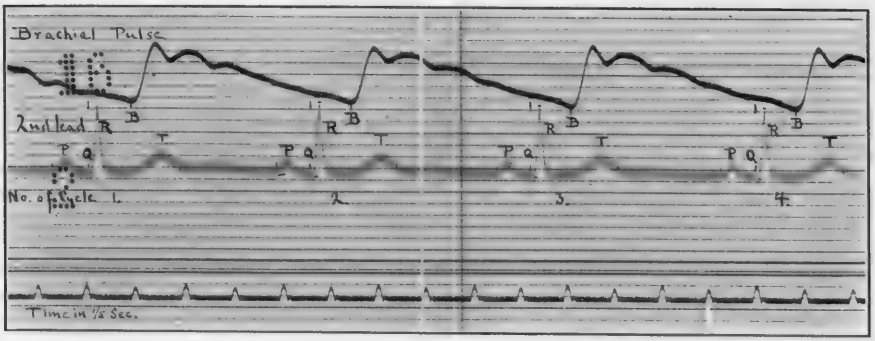
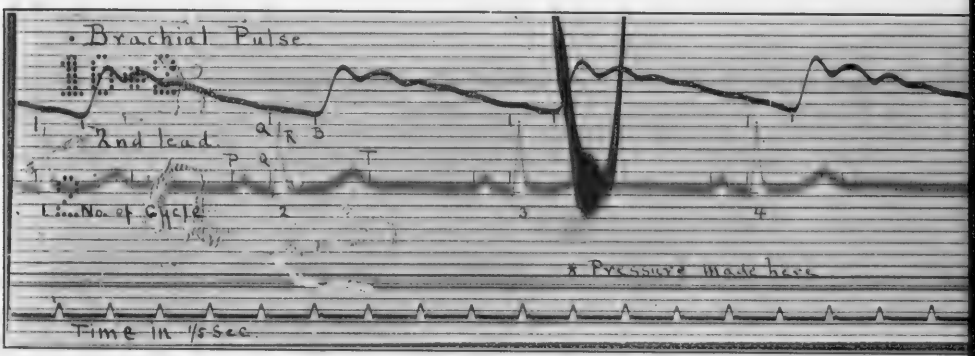
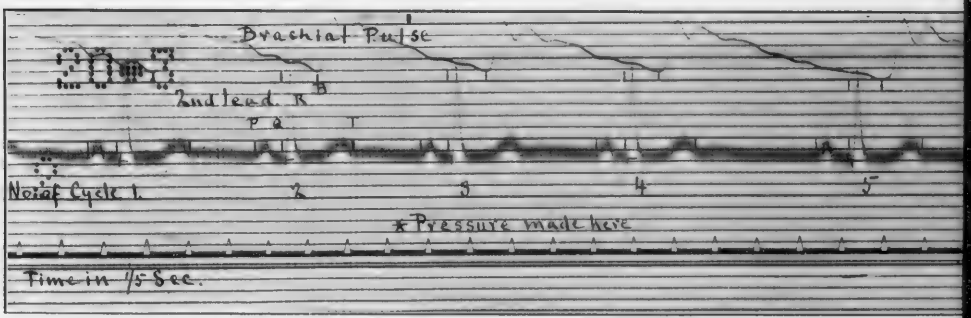
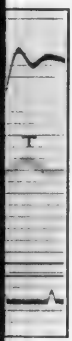

Brachial Pulse
2nd lead
R
B
P
Q
T
No. of Cycle 1.
2.
3.
4.
Time in 1/5 Sec.

FIG. 1.


Brachial Pulse
2nd lead
Q
R
S
P
T
No. of Cycle
1
2
3
4
* Pressure made here
Time in 1/5 sec.


Brachial Pulse
2nd lead
R
B
P
Q
T
No. of Cycle 1.
2
3
4
5
* Pressure made here
Time in 1/5 Sec.



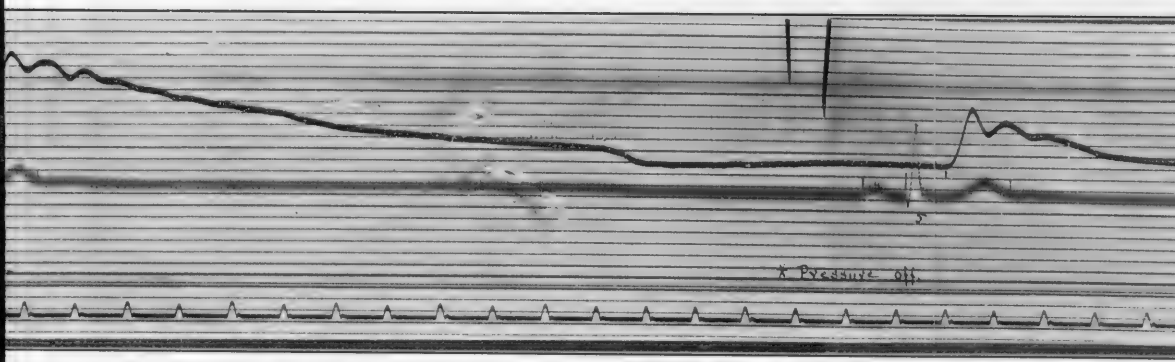


FIG. 2.

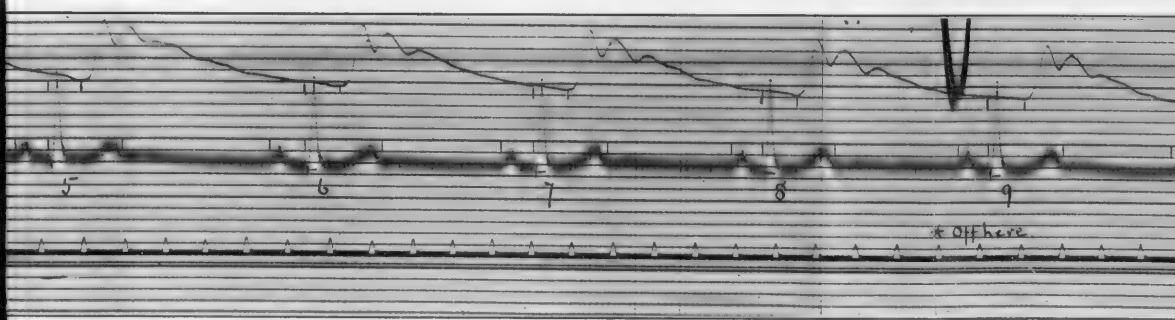
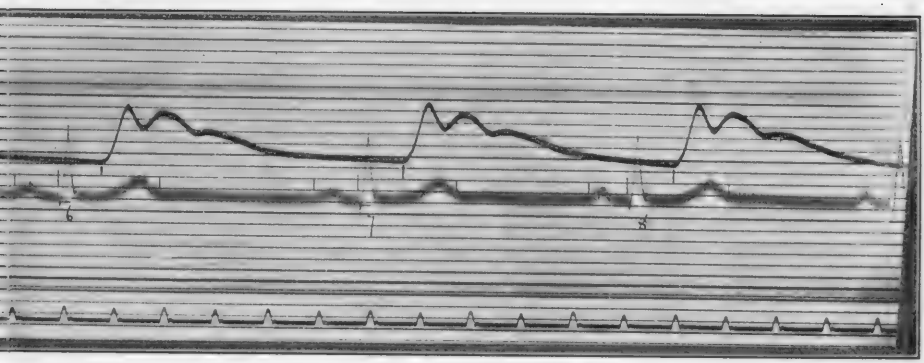
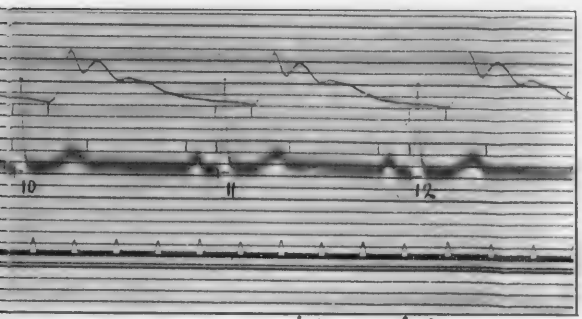


FIG. 3.

PLATE 22.





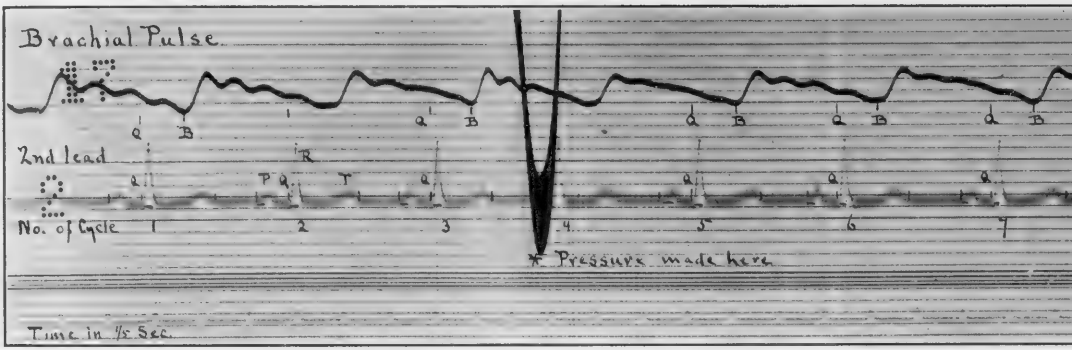
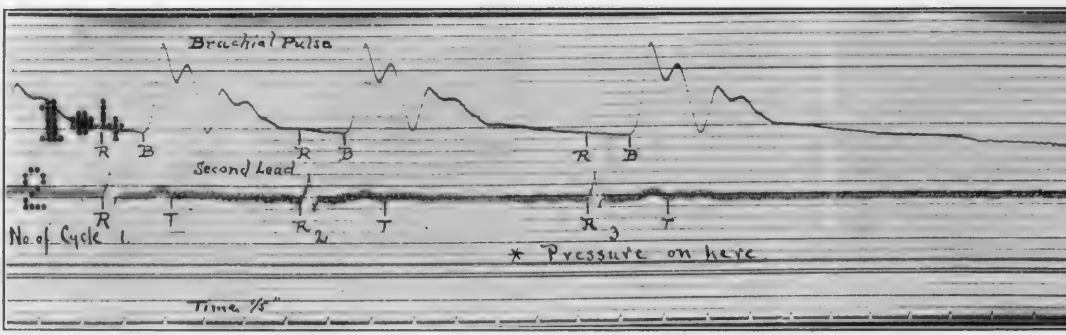
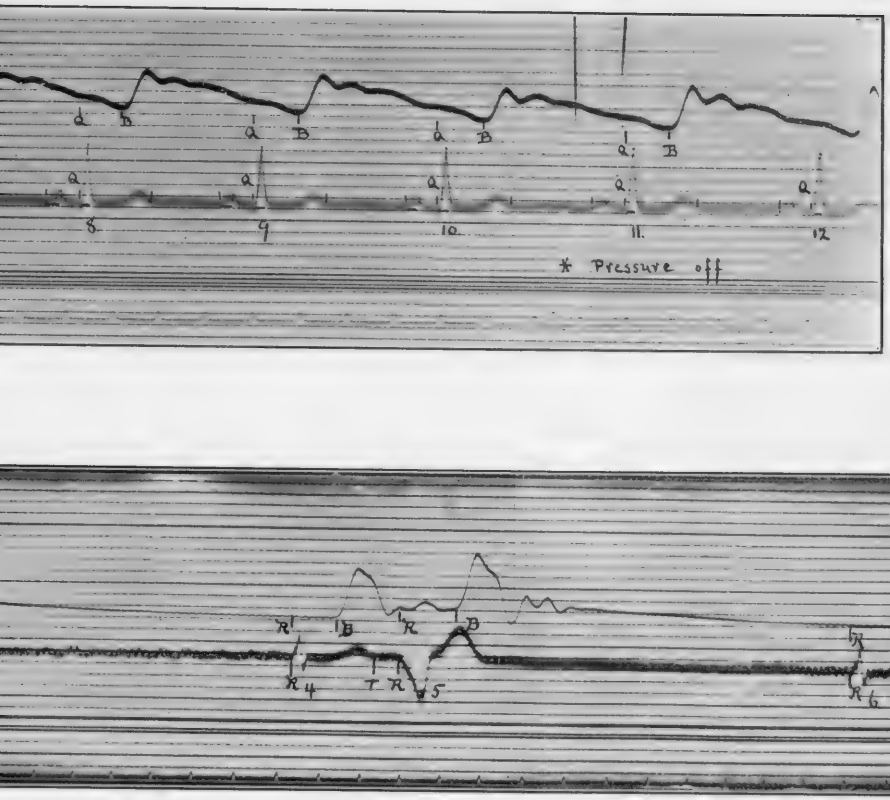

Brachial Pulse.
2nd lead
No. of Cycle
1
2
3
4
5
6
7
* Pressure made here
Time in 1/5 Sec.

FIG. 4.


Brachial Pulse
Second Lead
No. of Cycle 1
2
3
* Pressure on here
Time 1/5"



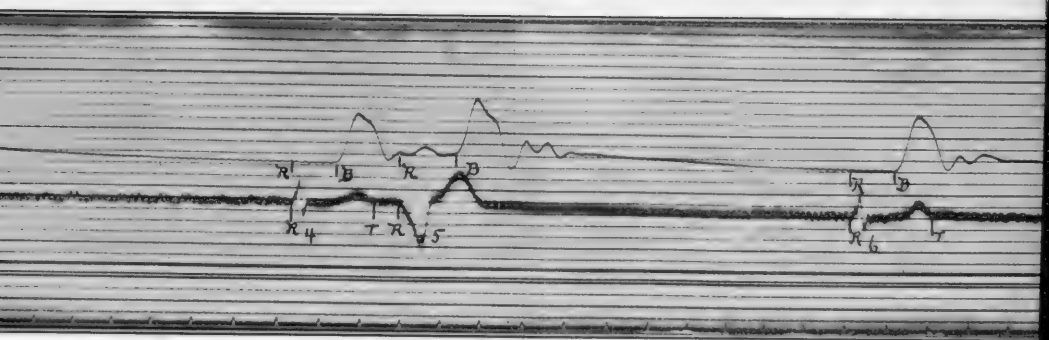
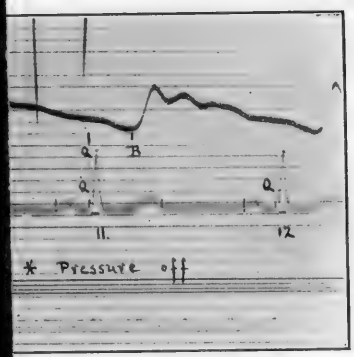
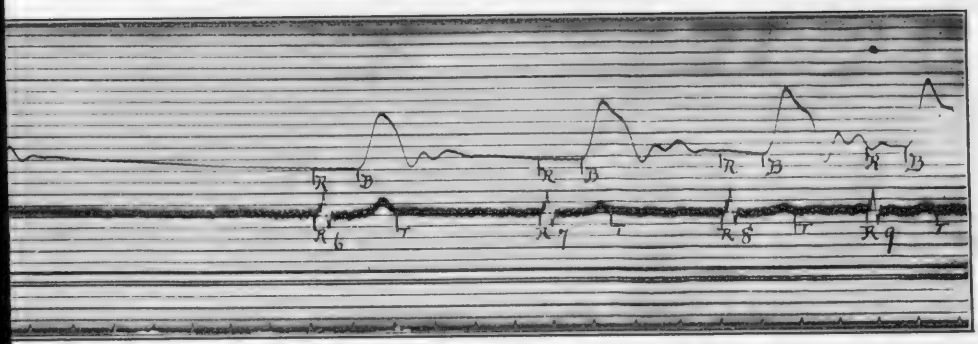


FIG. 5.

PLATE 23.





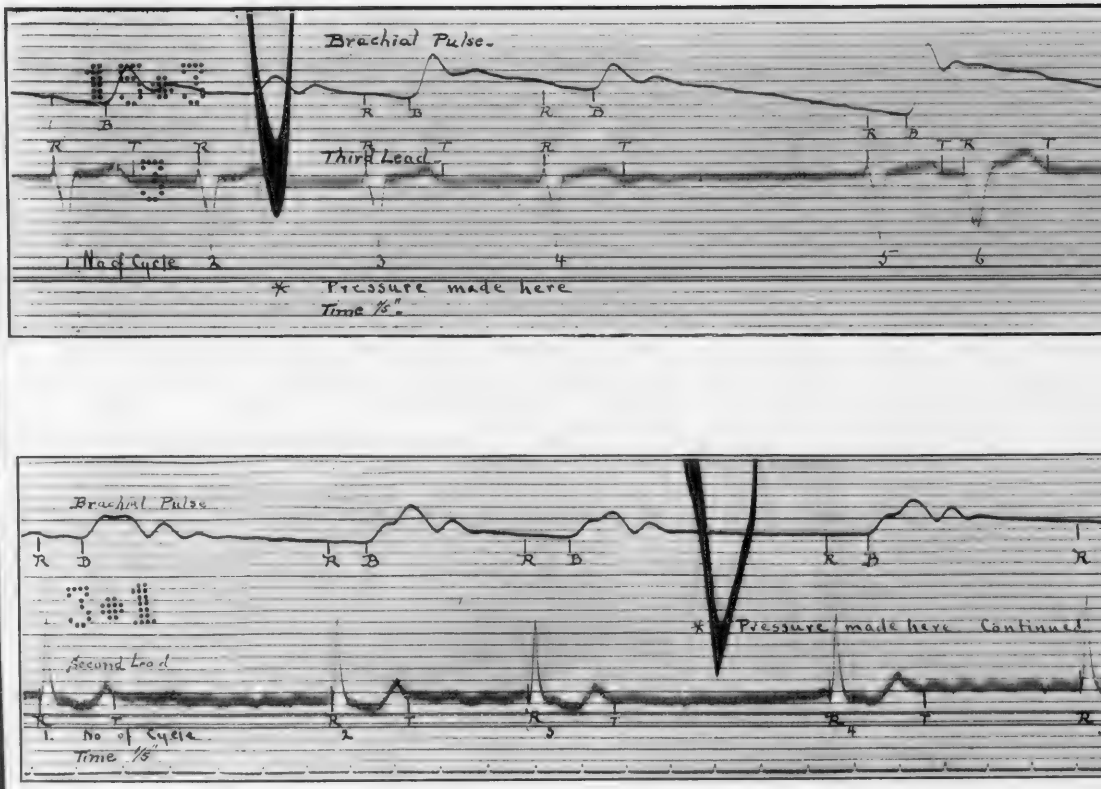

Brachial Pulse.
Third Lead.
1 No of Cycle
Pressure made here
Time 1/5".
Brachial Pulse
Second Lead
Pressure made here Continued
1. No of Cycle
Time 1/5"

FIG.

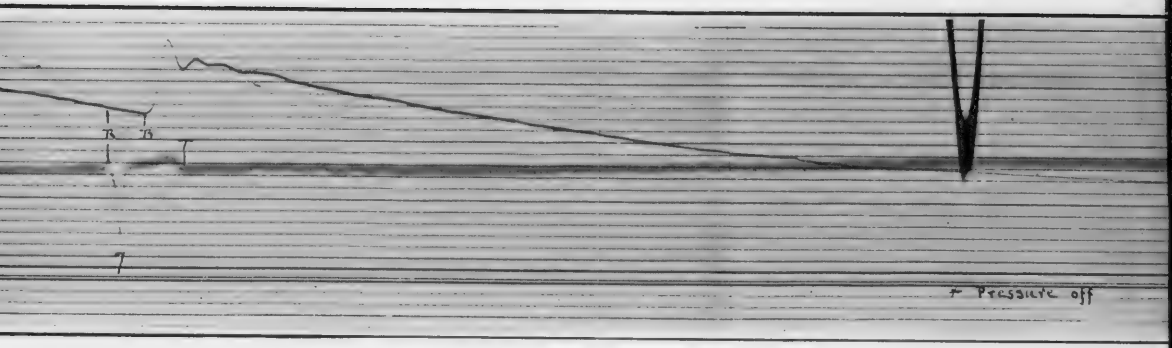
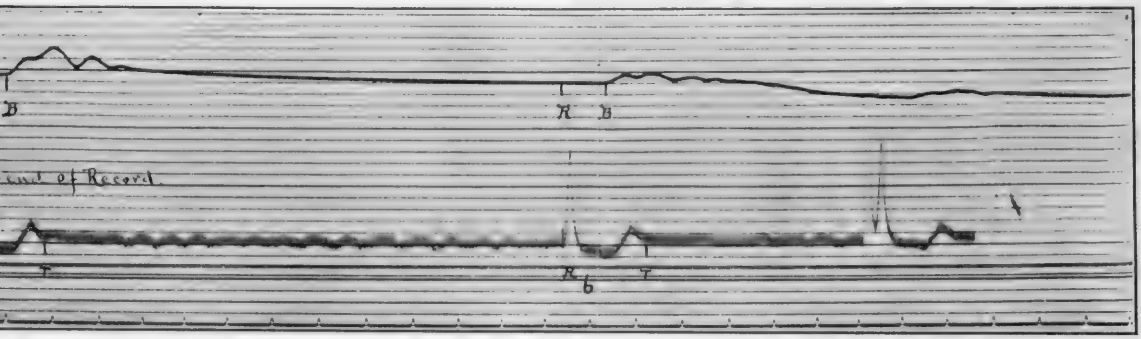
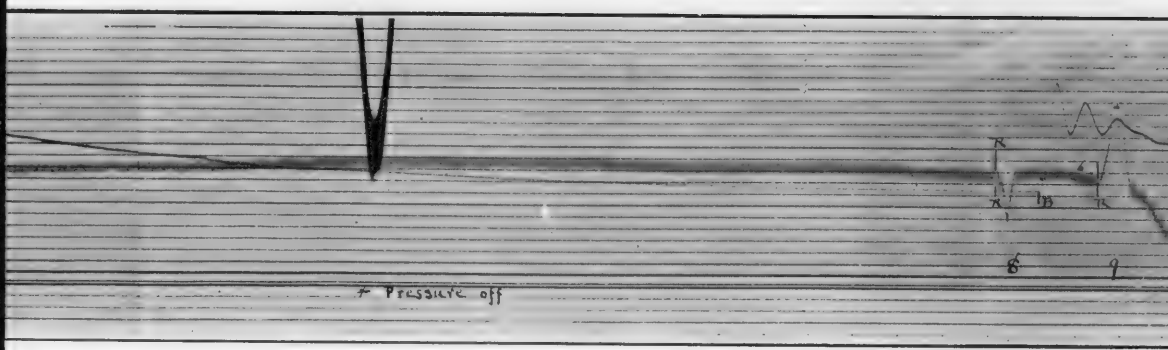


FIG. 6.




Pressure off

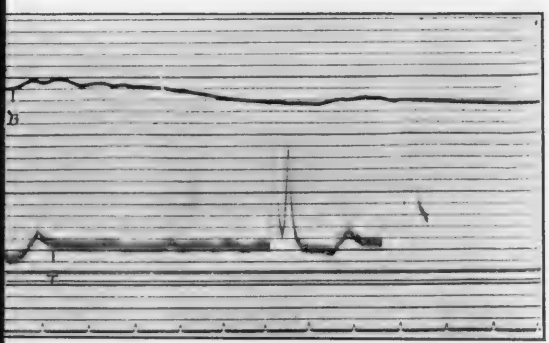
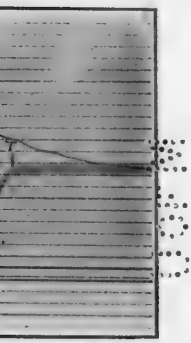


PLATE 24.



COMPLEMENT DEVIATION IN SCARLET FEVER WITH COMPARATIVE STUDIES OF THE WASSERMANN AND NOGUCHI SYSTEMS.*

By JOHN A. KOLMER, M.D.

(*From the Laboratory of the Philadelphia Hospital for Contagious Diseases.*)

Scarlet fever is a specific infectious disease of unknown etiology, running a definite course and conferring a specific immunity in the great majority of cases. This report is part of a series of investigations concerning the immune bodies, which it is believed must occur in the blood of persons having recovered from this infection.

Complement deviation tests were first undertaken with scarlet fever sera not with the hope of studying these hypothetical immune bodies, but to ascertain if the antigen prepared from luetic liver then in use would unite with something in the serum, deviate the complement, and thus render the sero-diagnosis of syphilis less reliable.

Much and Eichelberg first investigated the subject and examined the sera of twenty-five cases of which ten gave deviation or were "positive." The antigen employed by them—a watery extract of luetic liver—was evidently defective and their results misleading, but as the report appeared at a time when the Wassermann test was attracting attention, it stimulated interest because of its bearing on the serum diagnosis of syphilis. Later reports of other investigators showed considerable variation.

Of thirty-three cases examined by Jochmann and Töpfer only one case was positive, and this one proved negative at a later examination. Halberstaedter, Müller and Reiche examined ten cases, using several antigens prepared from luetic livers. The various antigens gave different results: five sera were weakly positive with one and negative with another. The remaining five were all negative. Boas and Hauge studied sixty-one cases; half before the disappearance of the rash and the other half at other periods. The antigen used was Michaelis' alcoholic extract of normal human heart. In three cases there was inhibition of hemolysis. In one, a girl nine years of age, who presented no evidences of syphilis, hemolysis occurred on the fiftieth day of illness. Another test fourteen days later proved negative. They concluded, "that complement-fixation in scarlet fever occurred so rarely and disappeared so rapidly after the illness that it can in no way hinder the great practical diagnostic worth of the Wassermann reaction."

*Read before the Philadelphia Pathological Society, June 8, 1911. Received for publication, June 13, 1911.

Bruck and Cohn examined twenty-eight cases, using as antigen eight different alcoholic extracts of syphilitic livers and one alcoholic extract of guinea-pig heart. Seven sera were positive with the syphilitic liver antigens and one with the heart antigen. The remaining twenty sera were negative. Meier tested fifty-two cases with a watery extract of luetic liver. In only one case was a slight positive reaction secured. Schleissner examined twenty cases with an alcoholic extract of guinea-pig heart as antigen and found all negative. Seligmann and Klopstock tested thirty cases by Michaelis' method. Their cases fell into two groups. The first fourteen yielded negative results. Cases 15 to 17 were weakly positive; cases 18 to 30 were strongly positive. Their results led them to state that complement deviation was of aid in the diagnosis of scarlet fever. Yet they must have suspected their antigens, because they say: "If an alcoholic extract can suddenly give a positive reaction with normal serum it is a fact of great theoretical and practical importance. . . ." Hoehne tested fifty-three sera from thirty-seven cases with an alcoholic extract of syphilitic liver. All were negative. Fifteen patients were examined twice and one three times to exclude a positive reaction late in the disease. Haendel and Schultz examined forty-eight cases in the same manner. Seven were positive and forty-one negative. Thirty-one of these sera were tried with a watery extract of scarlet fever liver and twenty were found positive. Fraenkel and Much studied twelve cases in the same way. Five yielded positive results; three were during the first week of the scarlet fever. Zeissler tested forty-two cases with an alcoholic extract of human heart. The cases were in the first to the forty-fourth day of the disease. As three cases gave positive reactions the author agreed with the statement of Much and Eichelberg that scarlet fever is more likely than any other disease to yield positive Wassermann reactions. Fua and Koch, who studied fifty-seven cases with an alcoholic extract of guinea-pig heart, found fourteen with positive, and forty-three with negative reactions. Finally, Hecht, Lateiner and Wilenko examined 105 sera, of which ninety-four were taken during life and eleven after death. In fourteen cases, the reaction was done twice. The ages of the patients varied from two to forty years; the examinations embraced all stages of the disease. As antigens, they used alcoholic extracts of guinea-pig heart and scarlet fever liver. Of the ninety-four sera from living patients they observed complement deviation in one case with the heart antigen and none with the scarlet fever liver antigen. The one positive result was in a case of severe nephritis. Of the eleven sera secured from patients after death, two were positive, in both of which nephritis was present. They conclude that complement fixation occurs in only a few sporadic cases of scarlet fever, that an antigen of scarlet fever liver is not specific, as Haendel and Schultz believed, and that the practical value of the Wassermann test in syphilis is not impaired by a few positive results in scarlet fever.

Apparently all of the work upon complement deviation in scarlet fever according to the Wassermann technique has been done by German investigators. Noguchi examined sera from sixty-two cases of scarlet fever according to his special technique with two doubtful positive and sixty negative reactions. The results secured

by the German observers varied from 40 per cent. positive reactions by Much and Eichelberg and 53 per cent. positive reactions by Seligmann and Klopstock to completely negative results by Schleissner, Hoehne, and others. Haendel and Schultz secured twenty-four positive reactions with thirty-one sera examined when using an extract of scarlet fever liver, and believed they had discovered an important fact, while Hecht, Lateiner and Wilenko after examining 105 sera with negative reactions in 102, concluded that such an extract was not specific and acted as an extract of guinea-pig heart or luetic liver. It is probable that many of these investigators secured false reactions by using faulty antigens—antigens that were themselves anticomplementary.

EXPERIMENTAL PART.

Sera from 250 cases of scarlet fever, representing all stages of the disease, were examined according to the following plan:

1. According to the Wassermann system with the following antigens:
   - (*a*) An alcoholic extract of luetic liver.
   - (*b*) An alcoholic extract of scarlet fever liver (from a malignant case of six days' duration).
2. According to the Noguchi system with:
   - (*a*) Active serum.
   - (*b*) Inactive serum.

The two following antigens were used with the active and inactivated serum of each case:

- (*a*) An alcoholic extract of luetic liver.
- (*b*) Aceton insoluble lipoids.

3. Because of the very important relation which the anti-sheep hemolysin normally present in human blood bears to the Wassermann system, the serum of each case was examined quantitatively for the presence of this hemolysin.

The 250 cases examined can be analyzed as follows:

Scarlet fever simplex	235 cases.
Scarlet fever malignant	13 cases.
Scarlet fever from burns of body	1 case.
Scarlatiniform serum rash	1 case.

During the first week or ten days of scarlet fever, the rash is present and begins to fade; during the second week, desquamation is usually well marked and is completed by the sixth or seventh week. Serum from the cases was secured at the following periods of the disease:

During the 1st week	58 cases.
During the 1st to 2d week	55 cases.
During the 2d to 3d week	32 cases.
During the 3d to 4th week	31 cases.
During the 4th to 5th week	28 cases.
During the 5th to 6th week	29 cases.
During the 6th to 7th week	11 cases.
During the 7th to 8th week	2 cases.
During the 8th to 9th week	4 cases.

Thirty-three patients had in addition to scarlet fever various complications, as follows:

Adenitis—cervical	3 cases.
Diphtheria—tonsillar	5 cases.
Nephritis—acute diffuse	7 cases.
Otitis media—suppurative	10 cases.
Pertussis	1 case.
Pharyngitis—pneumococcic	2 cases.
Pneumonia—broncho	3 cases.
Vincent's angina	2 cases.

The patients varied in age from one to thirty-five years.

WASSERMANN SYSTEM.

Technique.—Serum was inactivated at 55° C. for twenty-five minutes and used in amounts of 0.2 c.c. The antigens of luetic and scarlet fever liver were made after the method of Michaelis and Lesser, and every week or ten days were examined for exact antigenic value and for anticomplementary and hemolytic action in amounts of at least three antigenic units. The amount of luetic liver antigen used varied between 0.08 and 0.1 c.c.; the scarlet fever liver antigen between 0.25 and 0.3 c.c.; of anti-sheep hemolysin (rabbit serum) 0.002 c.c. represented two units. The pooled serum of two or more guinea-pigs was used as complement after titration for exact strength. Syphilitic and normal serum controls were used, with and without antigen. Serum, complement, antigen, and 0.85 per cent. salt solution up to 5 c.c. were mixed and incubated for one hour at 37° C.; 2 units of hemolysin and 1 c.c. of a 5 per cent. suspension of sheep corpuscles were then added and the whole incubated for two hours more, after which the tubes were placed in the refrigerator for twenty-three hours and the results noted.

Results.—(*a*) With alcoholic extract of luetic liver used as antigen: there were five, or 2 per cent., positive reactions out of the 250 sera tested. In these five cases positive reactions also resulted with scarlet fever liver antigen and with active and inactivated serum of the Noguchi system. The following are the five positive cases:

No. 1, Case 38, Mrs. B. A., 30 years. Fourteenth day of scarlet fever. After fading of the rash, she developed a peculiar eruption over the body a few days before these tests were made. History of luetic infection negative. All reactions were absolute or ++++, according to Citron's method of recording. No other complications.

No. 2, Case 87, R. H., male, 11 years. Thirteenth day of scarlet fever. Poorly nourished; no definite evidences of syphilis. All reactions slightly positive. No complications.

No. 3, Case 135, E. S., female, 6 years. Thirteenth day of scarlet fever. No evidences of syphilis. All reactions slightly positive. No complications.

No. 4, Case 150, M. D., female, 14 years. Twenty-eighth day of scarlet fever; sickly and undersized. All reactions moderate in degree. No complications.

No. 5, Case 160, Mrs. B. S., 23 years. Fortieth day of scarlet fever. History unsatisfactory but apparently negative. All reactions of moderate degree. No complications.

(*b*) With an alcoholic extract of scarlet fever liver used as antigen: the 250 sera were tested with this antigen. The antigen itself was tested with a large number of normal and syphilitic sera and was found to be antigenic in dosage from 0.25 to 0.3 cubic centimeter, and free of anticomplementary and hemolytic action. The results were practically identical with those resulting from the use of the luetic liver antigen. Deviation of complement resulted in six cases, or 2.4 per cent. These included the five cases given above with the addition of case 89, which also gave positive reactions with active and inactivated serum, according to the Noguchi system.

Case No. 89, M. H., male, 5.5 years. Eighth day of malignant scarlet fever. No definite evidences of luetic infection. No complications beyond the severe streptococcic angina of scarlet fever.

It is impossible to express any opinion as to whether any or all of these cases were syphilitic. The fact that in all the cases the other reactions were likewise positive lends some color to the probability of their being syphilitic, especially case 38. However, since but 2 per cent. of the scarlet fever cases were found to deviate complement with the regular Wassermann technique, we regard this reaction in scarlet fever *per se* as being uniformly negative.

As was expected, the antigen of scarlet fever liver acted in the same manner as that of the luetic liver. Hence our results agree with those of Hecht, Lateiner and Wilenko.

NOGUCHI SYSTEM.

Technique.—With active serum one drop (0.02 c.c.), and with inactivated serum four drops (0.08 c.c.) from a capillary pipette were used. Of the two antigens employed, one was an alcoholic extract of luetic liver in a dose of about 0.08 c.c., and the second of aceton insoluble lipoids, 0.1 c.c. of a 0.3 per cent. emulsion being used in each test. The antigens were examined every week or two for antigenic value, and hemolytic and anticomplementary effects. Anti-human hemolysin was used in such strength that 0.05 c.c. represented two units.[1] For complement, the pooled serum of two or more guinea-pigs in 40 per cent. strength was used after titration. The dose was usually about 0.1 c.c. Positive and negative controls with and without antigen were set up. Serum, antigen, complement, and 0.85 per cent. salt solution to make the total 1 c.c. were incubated at 37° C. for one hour. Then two units of hemolysin and 0.1 c.c. of a ten per cent. suspension of washed human corpuscles were added and the mixture was reincubated for two hours. Tubes were then placed at room temperature for an hour, and the results noted.

Results.—(*a*) With active serum there were sixteen, or 6.4 per cent. positive reactions. Five of these were with the five cases yielding positive Wassermann results. In five, or 2 per cent. of the cases (nos. 31, 50, 75, 81, 107), the reaction was positive with active serum alone, and negative with inactivated serum by both the Noguchi and Wassermann systems. In six cases the reactions were positive with both the active and inactivated serum. In other words, with the Noguchi system there occurred eleven more positive reactions than with the Wassermann system, five of these being with active serum alone.

(*b*) The first ninety of these reactions were made with alcoholic extract of luetic liver used as antigen, and it is well to note that four of the positive reactions which were obtained with active serum were made with this antigen. The remaining 140 cases were tested with an antigen of aceton insoluble lipoids from which but one positive reaction with active serum resulted.

[1] I am indebted to Dr. A. P. Hitchens and Dr. J. T. Ullom, who kindly gave me good hemolysin at a time when the sudden death of several rabbits would have stopped the work for a few weeks.

Noguchi has emphasized the danger of securing positive results with normal serum when using such an antigen as an alcoholic extract of liver, due to the fact that nucleoproteids, pepton, and various decomposition products of proteids in general are able to fix complement. The antigen of aceton insoluble lipoids, which is perfectly antigenic and neither anticomplementary nor hemolytic, at least in an amount of four antigenic units, has given entirely satisfactory results.

None of the eleven cases yielding positive results with the Noguchi system alone showed any evidences of syphilis, although it is, of course, a difficult matter to exclude the possibility of hereditary taint. From the fact that only one out of 140 cases, or 0.72 per cent., deviated complement with active serum in the presence of a satisfactory antigen of aceton insoluble lipoids, and in view of the fact that normal active serum will, in a small percentage of cases, deviate complement, I am inclined to conclude that in scarlet fever *per se* the Noguchi reaction is uniformly negative.

One of the main objections to the Wassermann system is the fact that there is present normally in human serum varying amounts of anti-sheep hemolysin. This is of great importance because an excess of the hemolysin can obscure a result by producing hemolysis in the presence of syphilitic antibodies up to a certain limit, even though less than one unit of complement be present. Practically all investigators have observed this effect, but very few have expressed their results in accurate terms. The 250 sera were tested by us for anti-sheep hemolysin, 0.1 cubic centimeter of undiluted guinea-pig serum being used in each test for complement.

Technique.—0.2 c.c. of inactivated serum, plus 0.1 c.c. of fresh guinea-pig serum, plus 1 c.c. of a 5 per cent. suspension of washed sheep corpuscles, plus 4 c.c. of 0.85 per cent. salt solution were employed. The incubation period was at 37° C. for one hour, followed by sixteen hours in the refrigerator, after which the results were noted.

Results.—Complete hemolysis occurred in 229 cases, or 88.1 per cent., and partial hemolysis in twenty-one cases, or 11.9 per cent. The amount of complement, 0.1 cubic centimeter of guinea-pig serum, was employed because this is the usual dose recommended, although serum from different animals varies in its complement

content. It emphasizes the necessity of careful titration of complement, for an excess of this factor, up to a certain limit, will give hemolysis with a decreased amount of hemolysin. The presence of anti-sheep hemolysin in normal human serum probably accounts for the negative Wassermann reaction with serum containing small amounts of syphilitic antibody. On the other hand, normal active serum will give a positive Noguchi reaction. For these reasons, we are led to agree with Kaplan that a Wassermann and Noguchi reaction should be performed with each serum—the one acting as a control upon the other.

CONCLUSIONS.

The Wassermann reaction in scarlet fever *per se* is uniformly negative. The antigen of scarlet fever liver yields practically the same results as that of luetic liver, and both fail to deviate complement with scarlet fever antibodies.

The Noguchi reaction in scarlet fever is practically negative. Sixteen cases, or 6.4 per cent. of 250 cases, were positive when active serum was used; with inactivated serum, but eleven, or 4.4 per cent., remained positive. Five of these eleven cases were also positive with the Wassermann system. In other words, sixteen cases, or 6.4 per cent., were positive according to the Noguchi system with active or inactivated serum or both, whereas with the Wassermann system only 2 per cent. were positive.

The presence of anti-sheep hemolysin normally in human serum is one of the main disturbing factors in the Wassermann system; for this reason, complement and hemolysin (made by immunization of rabbits) require careful titration. A positive Wassermann reaction usually indicates the presence of syphilitic antibodies, and a negative Noguchi reaction, their absence, and both systems should be used in the examination of all cases.

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AN ADDITION TO THE TECHNIQUE OF THE CULTIVATION OF TISSUES IN VITRO.*

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Plates 25 to 27.

In a previous article,[1] we have described a technique which permits of the growth *in vitro* of adult and embryonal tissues of mammals and other warm and cold-blooded animals. In this article, we shall mention briefly some modifications or improvements of the technique, relating to the medium of growth and the manner of preparation of the tissues and the cultures.

THE MEDIUM.

We have employed two kinds of media; namely, natural and artificial, so called.

The natural media were solid or fluid, consisting either of coagulated plasma or fluid serum.

Coagulated plasma has always given the best cultural results. The plasma can be kept in a fluid condition in cold storage for a long time. We have found chicken plasma, after having been preserved for two months, to be still an excellent culture medium. The use of the plasmatic medium is, therefore, very convenient. Autogenic and homogenic plasma constitute the best media, but heterogenic plasma can also be used, as has been shown by Lambert and Hanes.[2] For instance, in our experiments fetal tissues of the chicken grew extensively in human, rabbit, and dog plasma. In general, however, it may be said that the development of the tissues

* Received for publication, June 14, 1911.

[1] Carrel and Burrows, *Jour. Exper. Med.*, 1911, xiii, 387.

[2] Lambert and Hanes, *Jour. Am. Med. Assn.*, 1911, lvi, 587; *Jour. Exper. Med.*, 1911, xiv, 129.

is slighter, and often much less satisfactory in heterogenic than in autogenic and homogenic plasma.

We also employed normal plasma modified in different ways. Plasma diluted with two fifths of its volume of distilled water is more favorable to the growth of some tissues than is normal plasma. Ringer's solution, in the proportion of one part for one or two parts of plasma, or even in higher proportions, increases the rate and generally also the extent of the growth. A marked acceleration of growth has been observed when extracts of certain tissues, or heterogenic serum in which tissues have been allowed to autolyze, are used for diluting the normal plasma. The most extensive growths have been obtained in media of this type.

The natural fluid medium which we employed is serum. The growth is never as luxuriant in it as in the plasmatic media, for the reason that the cells find no support in the medium and hence must grow in a thin layer on the cover glass. Embryonal and tumor tissues grow well both in autogenic and homogenic sera; and an excellent growth may also be obtained in heterogenic serum. For example, the fetal tissues of the chicken grew extensively in rabbit serum, and equally well whether it was obtained from defibrinated blood or from coagulated plasma. Probably, therefore, the alexin is without essential effect on the growth of the cells.

The artificial media, so called, have given us positive results only as regards the cultivation of embryonal tissues or of tumors, and in these the growth was never as great as occurs in the natural media. The artificial media have been employed especially by M. R. and W. H. Lewis.[3] The medium advocated by them is composed of Locke's solution, agar, and bouillon. The one employed by us is composed of Locke's solution, to which 2 per cent. agar is added. We have not found that the addition of bouillon is an advantage. The proportion of salts can be altered in many ways, at least without necessary disadvantage. Tissues of the embryonal chick grow in .8 per cent. sodium chlorid solution, in Locke's and Ringer's solutions, and in various combinations of sodium chlorid, potassium chlorid, and calcium chlorid. In these media, the development goes

[3] Lewis, M. R., and Lewis, W. H., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 126; *Anat. Rec.*, 1911, v, 277.

on well for a short time; but neither the extent nor the duration of growth is nearly so great as in the plasmatic medium. Moreover, pure sodium chlorid solution is in itself harmful to the cells. This is shown by the fact that when fragments of chick embryos are well washed by maceration in large quantities of salt solution, they no longer grow even in plasma, while after a similar washing in Locke's solution, growth still takes place.

An important fact is the following: toluol can be added in the proportion of 4 per cent. to solid or fluid media, without apparent disturbance of the growth of tissues such as skin and nerve cells, while bacteria added in small quantities to the toluolized media do not multiply.

PREPARATION OF TISSUES AND CULTURES.

In our early experiments we employed tissues taken directly from the living animal. We found afterwards that tissues preserved in cold storage would grow. Spleen, skin, and heart of fifteen or sixteen day old chick embryos were kept for one day, two days, and even six days in cold storage before imbedding in the plasma and incubating. Although the latent period was markedly increased, the growth was characteristic.

For storing tissues, Ringer's solution may be employed, but not sodium chlorid solution, as it is toxic. Serum also is a less favorable medium of storage than Ringer's solution.

We first used the hanging drop culture and the larger cultures on plates; but more recently we have modified the technique with the view of obtaining better support for the cells and better nutrition for the cultures.

For the cultures in the plasmatic medium, watch glasses, sealed on glass plates, have been substituted for the ordinary cover glass. They are filled with a large quantity of plasma, or their internal surface is covered by a thin plasmatic layer. A fragment of spleen cultivated in plasma and serum has thus been kept alive for one month.

Fragments of tissue have been cultivated on pieces of silk veil impregnated with plasma (figure 1). It is a simple matter to cut the artificial tissue composed of living cells, plasma, and silk into

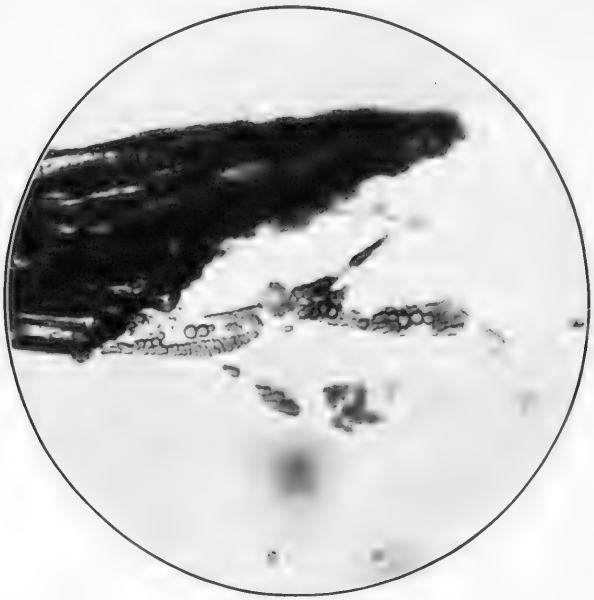

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

small pieces with the scissors, so that transplantation into a new culture medium can be effected without injury to the growing cells. The cells can also be seen growing from the silk veil into the culture medium (figure 2).

In the preparation of a culture in fluid medium, an important requisite is a suitable support for the growing cells. Although fibrin is an efficient support, it can be replaced by other bodies, such as cover glass, cotton, silk, etc. The cells grow in the fluid only if they meet with this condition of support. Therefore, the fragments of tissue must be very small in order to remain adherent to the cover glass, and the fluid must be spread out in a very thin layer about them. The growing cells cling to and spread over the surface of the glass (figure 3). But if cotton threads are placed in the fluid, the cells have a better support and grow luxuriantly around them (figure 4).

The conclusion is that the plasmatic medium is, beyond comparison, the optimum medium thus far studied for the growth of tissues. But cells can also grow in other media and under many different conditions. The technique of the cultivation of tissues *in vitro* is far from being worked out and a great many improvements will surely be developed.

EXPLANATION OF PLATES.

Plate 25.

Fig. 1. Skin of fetal chicken. Cells growing on silk veil and plasma. Peripheral part of a living culture, three days old.

Fig. 2. Skin of fetal chicken. Cells growing from a silk thread into a new medium. Living secondary culture, three days old.

Plate 26.

Fig. 3. Culture of fetal chicken skin grown in dog serum. Cells are clinging to the cover glass. Forty-eight hour culture. Stain, hemotoxylin.

Plate 27.

Fig. 4. A small part of a peripheral growth of a culture of fetal chicken skin grown in rabbit serum. Cells are clinging in part to the cotton thread and cover glass. Fifty hour growth. Stain, hemotoxylin.

THE EFFECT OF SALVARSAN UPON THE HEART IN DOGS.*

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PLATE 28.

During an investigation on the tolerance of salvarsan by rabbits, it was noticed that the blood pressure sometimes sank profoundly and abruptly during the intravenous injection of the drug, and the impression was produced that this was caused by a sudden weakening of the heart.[1] As there is, however, practically no experimental evidence that this drug does affect the heart, and as Nicolai[2] states definitely, on the basis of electrocardiograms, that salvarsan does not affect this organ, it seemed desirable to study this aspect of the problem more directly. For this reason the following series of experiments were carried out.

METHOD.

For reasons of convenience, dogs only were employed. Their weight varied from four to seven kilos. They were anesthetized by ether and kept under the influence of the anesthetic by means of intratracheal insufflation.[3] As a rule, curarin was given. A cannula was then introduced into the external jugular vein, the sternum split transversely and the heart fully exposed. After the pericardium was opened the ventricles were placed in an oncometer.

This oncometer was made from a bottle whose bottom had been cut away and replaced by a thick rubber dam. In this rubber dam an oval aperture was burned and the ventricles were pushed into the cavity of the glass chamber.

* Received for publication, June 20, 1911.

[1] Auer, *Arch. Int. Med.*, 1911, viii, 169.

[2] Nicolai, Kongress für innere Medizin, Wiesbaden, 1911, *Deutsch. med. Wchnschr.*, 1911, xxxvii, 859.

[3] Meltzer and Auer, *Jour. Exper. Med.*, 1909, xi, 622.

The junction between dam and auriculo-ventricular groove on the inside of the chamber was lined with cotton soaked in vaselin. The oncometer was then retained in position by a clamp fixed to a stand, and the chamber itself was connected with a Marey tambour.

In this fashion, the volume change of the ventricles was recorded. In addition, as a rule, the carotid blood pressure was written by means of a Hürthle membrane or spring manometer, the connecting tubing being filled with a 10 per cent. solution of sodium citrate.

The solutions of salvarsan were prepared in two ways, either alkaline or acid. When the alkaline solution was employed, one cubic centimeter of a normal solution of sodium hydrate was added for every 0.1 gram of the drug, which had previously been ground into solution in a mortar with a quantity of 0.9 per cent. saline solution. A perfectly clear yellowish green liquid resulted, which was then diluted with physiological salt solution to the desired concentration. For the acid solution the drug was merely ground in a mortar with gradually increasing quantities of 0.9 per cent. saline solution; this produced a clear yellow solution which was then diluted.

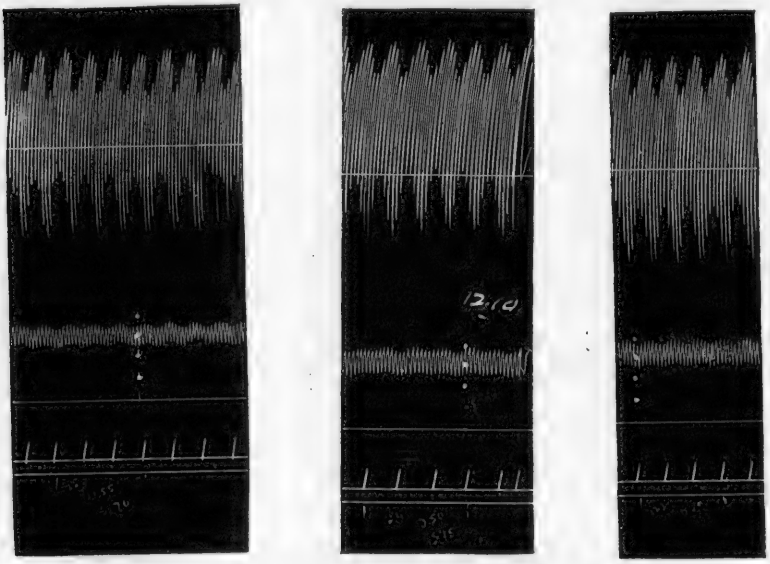
The concentration of the drug employed most often in this work was 0.5 per cent.

RESULTS.

Alkaline Solution.—The animals stood the infusion of salvarsan in 0.2 per cent. or 0.5 per cent. alkaline solution very well, even with the enormous doses infused (twenty to forty milligrams per kilo. of body weight). Text-figures 1 and 2 show this very well, and no marked changes of either heart volume, blood pressure, or heart rate are recorded. On the basis of this, it might be assumed that the heart had suffered very little, if at all. This impression, however, would be erroneous, as the following observations will show.

In the animal which furnished the curves for text-figure 1, thirty-six milligrams of salvarsan per kilo. in 0.2 per cent. alkaline solution had been infused in six minutes. The blood pressure and heart rate were practically unaltered after the infusion; the heart, however, showed a moderate dilatation which persisted for about twenty minutes after the infusion (text-figure 1, *a, b,* and *c.*). Thirty-five minutes after the infusion, the heart was apparently normal (text-

figure 1, *c*). But on removing the heart from the oncometer, and handling it very gently, it passed at once into fibrillation, from which it did not recover. Similar effects were observed in other experiments where the alkaline solution was infused. On the other



a *b* *c*

TEXT-FIGURE 1. April 5, 1911. Dog ♂ 4,400 grams; 36 mgm. per kilo., 0.2 per cent. *alkaline* solution into jugular vein. *a* = curve obtained 1 minute before infusion. *b* = curve obtained 6 minutes after infusion. *c* = curve obtained 24 minutes after infusion.

In all the tracings the upper curve represents ventricular volume; downstroke, systole. The horizontal line transecting this curve is the atmospheric pressure line.

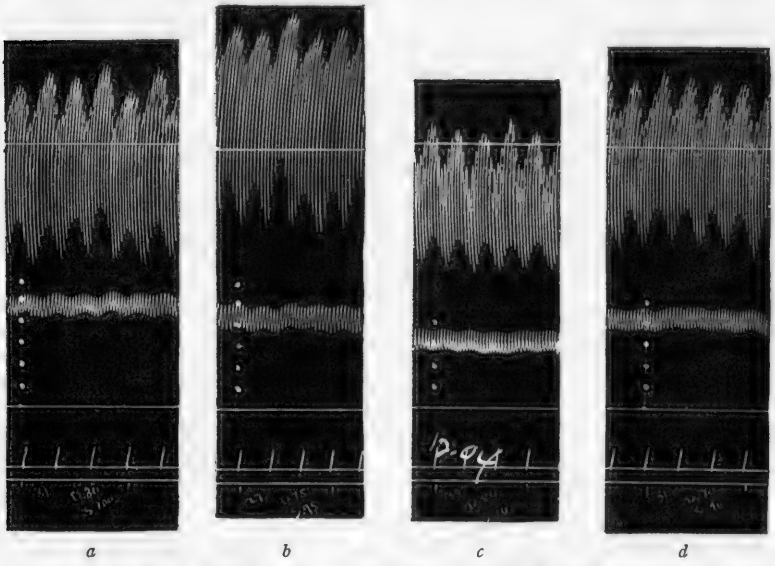
The second curve is the blood pressure obtained by a Hürthle manometer connected with the carotid artery; its value in mm. of mercury may be obtained from the calibration in 20 mm. intervals (shown by dots in figures 1 and 2). The straight line below the blood pressure curve is the zero pressure.

Time is marked in all tracings in four second intervals.

hand some hearts of the same series of experiments stood manipulation very well, and only a very transitory change in the heart's action was observed.

Acid Solution.—This solution was employed in 0.5 per cent. solution only because with this strength toxic symptoms could be easily obtained. With a weaker solution, slight or no toxic effects were noticed, which is in accord with observations made in rabbits, where it was found that the toxicity of acid solutions of salvarsan varied directly with the concentration.[4]

With an animal prepared for the registration of blood pressure and heart volume, as described above, the infusion of 0.5 per cent.



a *b* *c* *d*

TEXT-FIGURE 2. April 7, 1911. Dog ♂ 6,400 grams; 31 mgm. per kilo., 0.5 per cent. *alkaline* solution into jugular vein. *a* = curve just before beginning infusion. *b* = curve 2 minutes after infusion. *c* = curve 38 minutes after infusion. *d* = curve 64 minutes after infusion.

acid salvarsan produces swiftly a moderate dilatation of the heart; then the blood pressure begins to fall and reaches a low level rather promptly. During this fall, the pulse pressure diminishes rapidly, and at the lowest level of blood pressure the heart rate is very slow. After a low blood pressure has been reached, the heart

[4] Auer, *Arch. Int. Med., loc. cit.*

dilates still more and may even stop in diastole for a period of seconds (plate 28, figure 1). Then the heart begins to beat again, but the beats are weak and hardly affect the lever of the membrane manometer which is connected with the carotid artery. From this state the animal does not recover, though the heart, as a rule, keeps on beating feebly for a number of minutes.

Results similar to those described above were obtained in practically all of the ten experiments which were made with the 0.5 per cent. acid solution. Some apparent exceptions will be discussed later.

DISCUSSION.

In the preceding pages, experimental evidence has been given which shows that both alkaline and acid solutions in 0.5 per cent. strength may affect the heart. With the alkaline solution, this action is not so obvious as with the acid solution, nor is it as constant. Neither the blood pressure nor the volume changes of the heart form a safe guide with the alkaline solution, for it was shown above (text-figure 1) that both these indicators may be but moderately affected by as much as thirty-six milligrams of salvarsan per kilo., and yet a comparatively slight extra strain put upon the heart was sufficient to throw it into a fatal fibrillation. It must also be especially noted that this occurred with a weak solution,—only 0.2 per cent. Fortunately, however, this does not occur regularly, even when 0.5 per cent. is used, and, as stated before, the heart may then withstand severe treatment. This failure is probably due to an inherent weakness of the cardiac muscle which is brought out by the injection of salvarsan, and the contention of Ehrlich and many clinicians that myocarditis be regarded as a contra-indication for the intravenous injection of salvarsan has thus experimental support. This danger of sudden cardiac failure will always be present as long as the functional capacity, the factors of safety,[5] of the heart tested is not known, for it is well known that a heart may be perfectly able to meet all ordinary requirements, and thus seem normal, and yet these ordinary requirements may demand all that the heart is able to give; when an extraordinary occasion arises this heart is

[5] Meltzer, *Jour. Am. Med. Assn.*, 1907, xlviii, 655.

unable to respond, and is forced into fibrillation and diastolic stoppage.

When salvarsan is employed in 0.5 per cent. acid solution, the effect on the heart is obvious; the tracing in plate 28, figure 1, shows it better than a mere description could. This tracing shows that the profound weakening of the heart contractions is preceded by a marked drop in blood pressure. But this drop in pressure cannot be attributed entirely to a vasomotor disturbance, as is done by Hoke and Rihl,[6] for the volume record (upper curve in plate 28, figure 1) demonstrates that the ventricles are full of blood but are practically unable to expel their contents, at least as far as the left ventricle is concerned, and the carotid blood pressure manometer shows only slight or no pressure waves. Moreover, the dilatation cannot be attributed to a too rapid infusion, as only twenty-two cubic centimeters were injected and the incorporation of this small quantity consumed four minutes. These facts clearly indicate that an effect upon the heart itself is produced by acid salvarsan.

These two series of experiments, therefore, show quite well that salvarsan, either in alkaline or acid solution, may exert a harmful effect upon the heart. Whether this action is exerted upon the cardiac muscle, or upon the vagus endings, or upon both structures, cannot be definitely stated. The action seemed to be chiefly muscular, although in a few experiments, where the vagi were cut, it seemed as if the cardiac vagus endings became more irritable to faradic stimulation for a short period of time after the infusion of salvarsan.

Relative Toxicity of Acid and Alkaline Solutions.—The experiments which have been briefly described in the preceding pages showed strikingly that the 0.5 per cent. acid solution is much more toxic than the 0.5 per cent. alkaline solution.[7] With the acid solution, ten experiments were carried out, and eight of the dogs died during or shortly after the infusion. With the alkaline solution in 0.2 per cent. or 0.5 per cent., eight experiments were made. The dosage per kilo. was about the same in both series, ranging between 20 and 40 milligrams. All of these animals stood the alkaline solution well as far as blood pressure and ventricular volume were concerned, and no fatalities occurred within an hour,

[6] Hoke and Rihl, Kongress für innere Medizin, Wiesbaden, 1911, *Deutsch. med. Wchnschr.*, 1911, xxxvii, 859.

[7] J. and L. Camus, *Paris méd.*, 1910, i, 66. Hering, *München. med. Wchnschr.*, 1910, lvii, 2621.

after which the animals were usually killed. It has already been stated that manipulation of the heart of some of these animals caused marked changes and even stoppage, while the others recovered very promptly.

Relative Toxicity of Some Preparations of Salvarsan.—While experimenting with the acid solution of the drug, some evidence was encountered which seemed to show that different preparations of salvarsan do not possess the same toxicity. The earlier experiments with the acid 0.5 per cent. solution were chiefly made with preparation marked "606, 1/250," while in the later experiments, a preparation marked "Salvarsan Op. 130" was employed. With the acid 0.5 per cent. solution of "606" eight out of ten dogs died in a short time after a dose varying from 14 to 33 mgm. per kilo. "Salvarsan Op. 130" was used in the same concentration in four additional dogs. These received from 30 to 40 mgm. per kilo and none of them died; the blood pressure of all of these animals, three to four hours after the infusion, varied from 140 to 190 mm. of mercury, and they seemed to be in excellent condition. In three of these animals the vagi had been severed in order to study the cardiac vagus during the action of salvarsan; in the fourth animal these nerves were intact.

This difference in toxicity of the two preparations may, however, be due to the fact that the four dogs of the last series, where "Salvarsan Op. 130" was injected, were not subjected to the same severe operation as the dogs of the earlier series: merely the blood pressure was recorded, and no tracing of the heart volume was taken. It may be, therefore, that the animals were in better physical shape and could withstand the action of the drug better, and that there is no real difference in the toxicity of the two preparations. This explanation, however, is not adequate, for two experiments with "606" were carried out with dogs where no operation was performed beyond the introduction of a cannula into the external jugular vein. These animals received 20 mgm. of acid "606" in 0.5 per cent. solution. One died during the infusion and the other died twenty-five minutes after it. In both, the heart was no longer beating on immediate autopsy, and this organ was so dilated that it snugly filled the entire pericardial sac. These data show that "606" was surely more toxic than "Salvarsan Op. 130."

This varying toxicity must, therefore, be taken into consideration, especially as other observers have also described differences in the various preparations of the drug. Thus J. and L. Camus[8] report some apparent variations, and recently Hoke and Rihl[9] state that "hyperideal" is twenty times more toxic than salvarsan, and that these differences in toxicity are especially pronounced in the acid solutions.

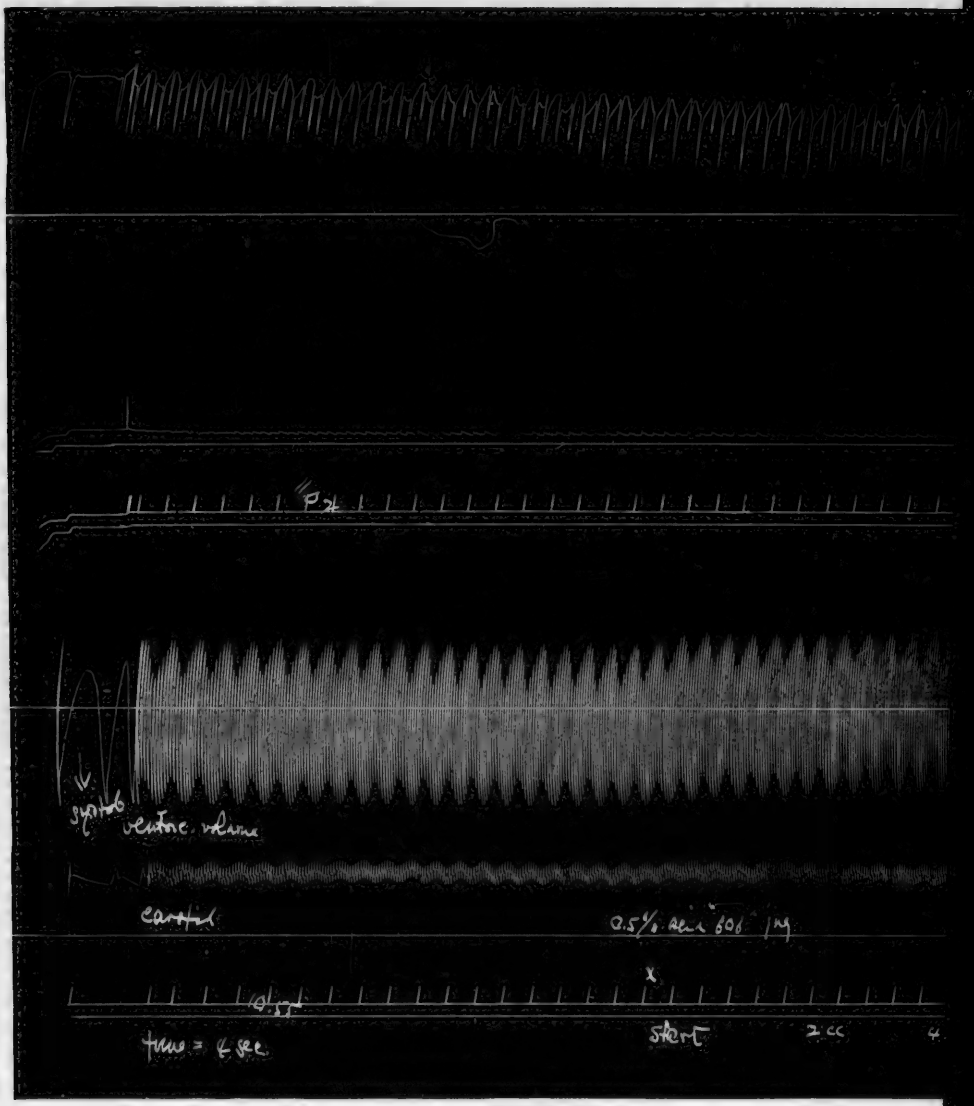
SUMMARY.

The intravenous infusion of 0.5 per cent. *acid* "606" causes a weakening of the heart which is usually fatal.

The intravenous infusion of 0.5 per cent. *alkaline* "606" causes no marked apparent effect upon the heart, but its factors of safety

[8] J. and L. Camus, *Paris méd., loc. cit.*

[9] Hoke and Rihl, *Deutsch. med. Wchnschr., loc. cit.*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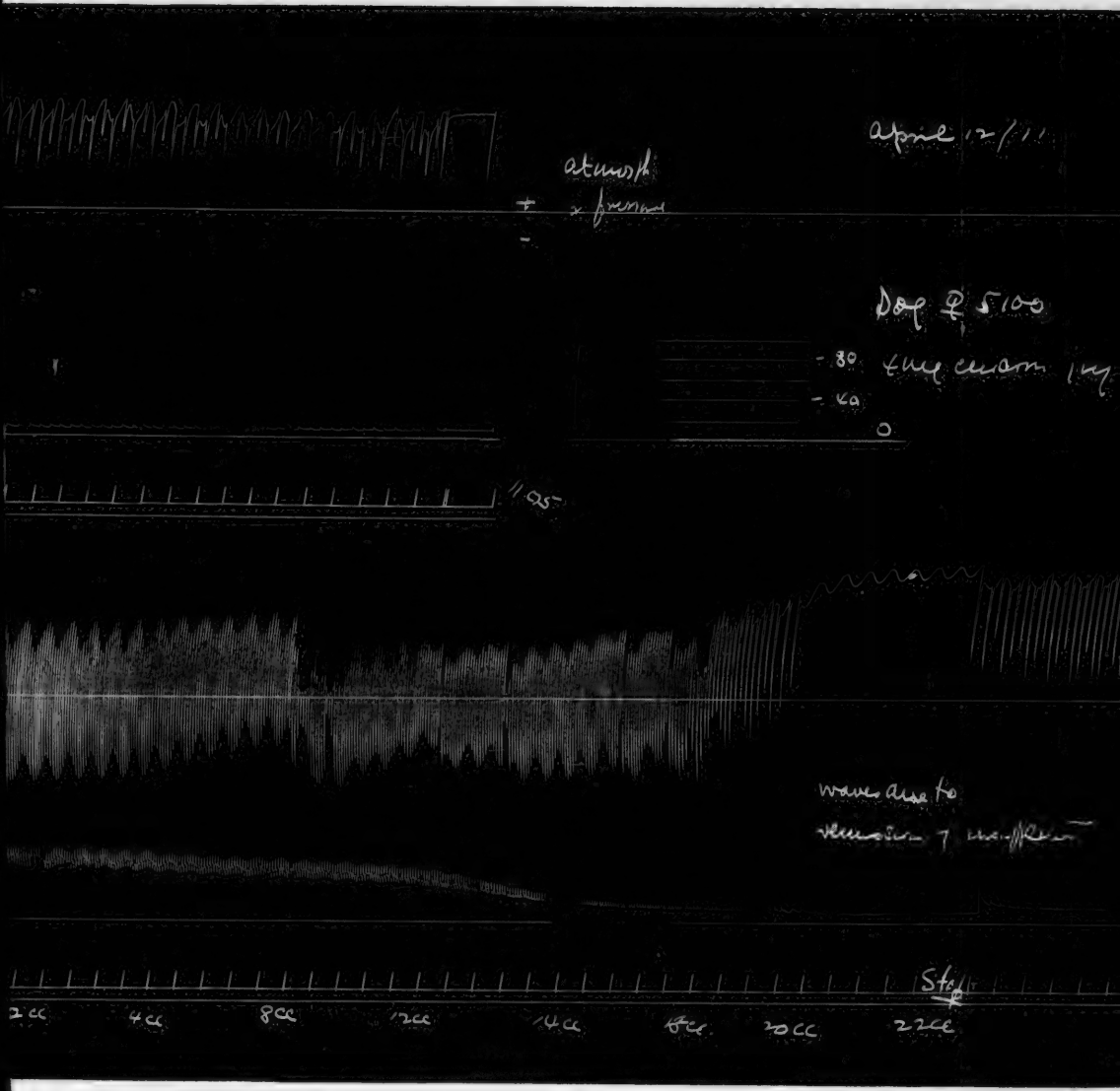
April 12/11
atmosph
+
-
Dog @ 5100
- 80
- 60
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2 cc
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8 cc
12 cc
14 cc
16 cc
20 cc
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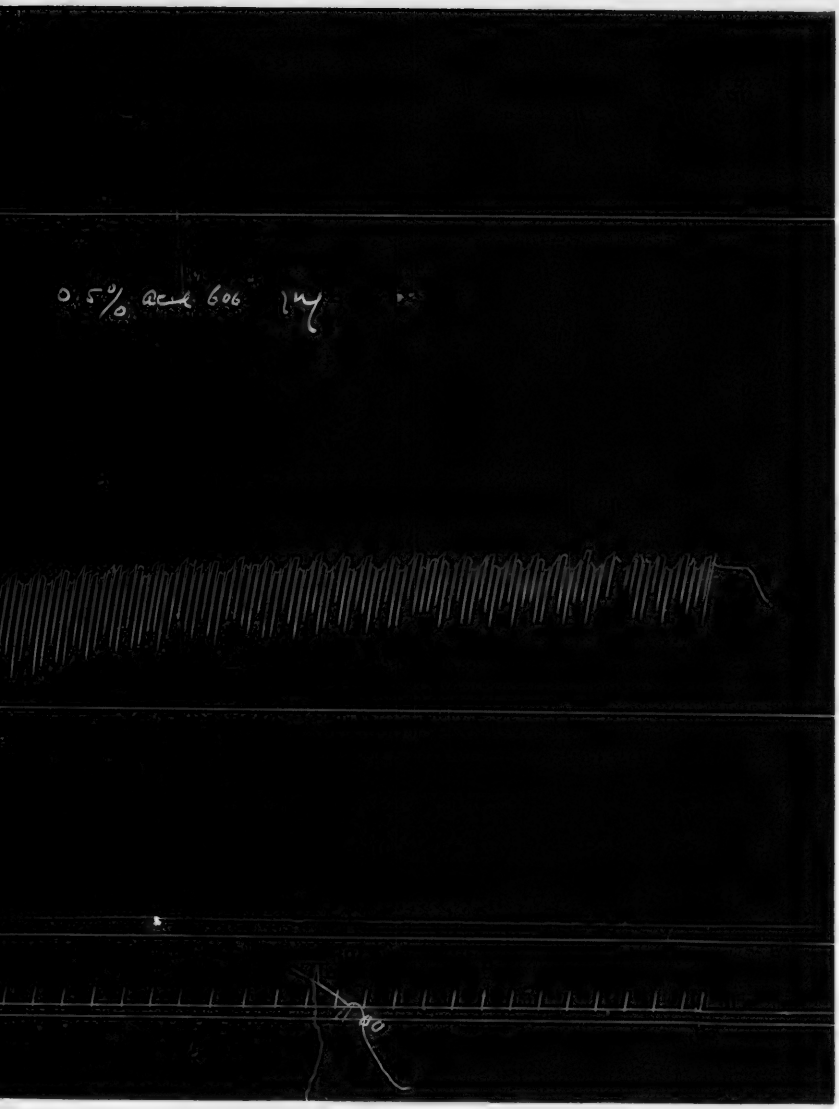
FIG. 1.

PLATE 28.



may be so reduced in some instances that a slight extra strain produces fibrillation and death.

EXPLANATION OF PLATE 28.

FIG. 1. April 12, 1911. Dog ♀ 5,100 grams; 21 mgm. per kilo., 0.5 per cent. *acid* solution into jugular vein.

The waves shown during the stoppage of the heart are caused by the remissions of pressure in the lung produced by the method of insufflation.

CHEMO-IMMUNOLOGICAL STUDIES ON LOCALIZED INFECTIONS.*

Third Paper: Some Further Observations upon the Action of Certain Soaps on the Pneumococcus and Its Experimental Infections.

By RICHARD V. LAMAR, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

As related in the first paper,[1] it has been found that sodium oleate injures, kills, and dissolves virulent pneumococci, and although its action in the test tube is inhibited by serum, it is possible under certain quantitative conditions of experiment to circumvent this serum inhibition by the use of boric acid and thus to employ successfully sodium oleate and immune serum conjointly in combating artificial pneumococcic infections. It was also stated that after inoculating susceptible animals with cultures of the pneumococcus, better results, in preventing fatal infection, were secured by treatment with combined sodium oleate and pneumococcus immune serum than with either alone. At the same time it was pointed out that there is a limit in the time which can be allowed to elapse between inoculation and treatment beyond which it has not been found possible to save the infected animal, although the treatment too long delayed to prevent death, usually defers the fatal issue.

Realizing, therefore, the limitations to the use of sodium oleate and yet recognizing in it an agent which possesses in some degree the three most important desiderata of an "internal" antiseptic remedy, *viz.*, great toxicity for the infecting parasite, little toxicity for the host, and ability to act in the presence of serum,—we have continued the studies in combating artificial pneumococcic infections in two directions. The one had for its object a widened field

* Received for publication, June 20, 1911.

[1] Lamar, *Jour. Exper. Med.*, 1911, xiii, 1.

of the therapeutic application of sodium oleate and immune serum, as well as the enhancing of the activity of the soap in the presence of serum; the other, a search for a more effective agent to substitute for sodium oleate. This paper deals with the latter phase of the problem, yet it may be said here that some encouraging results, to be published later, have been obtained in respect to the extension of the therapeutic use of combined sodium oleate and pneumococcus immune serum.

Comparing the strong bactericidal action of the soaps of oleic acid with the absolute inactivity of those of its analogue in the saturated series—stearic acid—we have in the fact of unsaturation and resulting chemical affinities a probable explanation of this activity and hence an indication of the direction in which the search for a substitute might well be begun, *i. e.*, among the more highly unsaturated fatty acids. Several such acids were obtained and tested as free acids and as alkali soaps. A summary report of their action on the pneumococcus in the test tube was made in the second paper,[2] where it was stated that potassium linoleate and linolenate were much more destructive to the pneumococcus than oleates and, also, that a given quantity of serum inhibited their action to a lesser degree. The present paper is concerned with the properties of these two acids.

Kahlbaum's linoleic and linolenic acids were used. The former had physical properties and an iodin value (181.4) indicating approximate purity; the latter an iodin value of only 243.2, indicating admixture of less unsaturated acid, partial "drying," or both. Yet considering the impossibility of obtaining the acid in a pure state and the great difficulty of obtaining it more nearly pure than Kahlbaum's, it was considered a satisfactory specimen for the purpose of this investigation. Potassium and sodium soaps were made in the usual way with alcoholic alkali solutions. The soaps are more freely soluble than the corresponding oleates and form in 10 per cent. concentration perfectly clear, limpid solutions. No difference was detected in the action of the potassium and sodium soaps of the same acid, and a difference only in intensity of action between the soaps of the two acids, the linolenate being about one half again as

[2] Lamar, *Jour. Exper. Med.*, 1911, xiii, 380.

potent as the linoleate. The comparative bacteriolytic strengths of oleate, linoleate, and linolenate may be expressed roughly by the ratios 1:4:6.

When the linoleate or linolenate soaps are added to a broth culture of pneumococcus so as to form a dilution of 1 to 1,000, and even 1 to 5,000, the pneumococci are killed and dissolved with great rapidity. At dilutions of 1 to 500 and 1 to 1,000 the rapidity and intensity of action are such that, as revealed by hanging drop and film preparations, the solution of the cocci is well under way in one minute and complete in a few minutes. With linoleate at 1 to 4,000 and linolenate at 1 to 6,000, the culture is sterilized and the cocci apparently completely dissolved within one hour at room temperature. When the dilution of soap is greater, solution of the cocci is correspondingly less, although it is still manifest at dilutions of 1 to 20,000 of linoleate and 1 to 30,000 of linolenate.

In a similar manner to that of sodium oleate, highly dilute solutions of these soaps change the pneumococci in some way, without killing or appreciably altering the form or staining properties, so that when washed and resuspended in salt solution or serum they disintegrate with great rapidity. In normal serum the disintegration is incomplete, and the cocci which escape destruction multiply later. In immune serum destruction is complete. This kind of injurious action is exerted by sodium linoleate in dilution of 1 to 60,000 and by sodium linolenate in dilution of 1 to 100,000.

Moreover, when pneumococci are so treated with the great dilutions of the soaps, washed and resuspended in salt solution, and injected alone and in combination with normal and immune serum into rats, all the animals die of pneumococcic septicemia except those which received the treated cocci and immune serum. The latter are at most transitorily ill. A protocol will serve to illustrate one of the experiments.

March 30, 1911. 12:30 P. M. Treated a 22 hours' plain broth culture of N. I. 66 with sodium linolenate at a dilution of 1 to 75,000 for one hour at room temperature; collected the bacteria and washed once with salt solution by centrifugalizing; resuspended the cocci in salt solution so that 0.5 c.c. of suspension represented 2 c.c. of the original culture. Washed and resuspended in the same way the normal diplococci contained in another portion of the same broth culture.

3 P. M. Injected white rats, in duplicate series, intra-abdominally; each rat receiving 0.5 c.c. of suspension or the equivalent of 2 c.c. of a culture of which 0.000001 c.c. is fatal for grown rats.

TABLE I.

	Mar. 31, 1911.	Result.
No. 1. Soaped cocci alone.	Rather ill.	Died 40 hrs. after inoculation; pneumococcus septicemia.
No. 2. Soaped cocci alone.	More ill.	Died 38 hrs. after inoculation; pneumococcus septicemia.
No. 3. Soaped cocci+normal serum, 0.2 c.c.	Very ill.	Died 36 hrs. after inoculation; pneumococcus septicemia.
No. 4. Soaped cocci+normal serum, 0.2 c.c.	Very ill.	Died 60 hrs. after inoculation; pneumococcus septicemia.
No. 5. Control, washed cocci+immune serum, 0.2 c.c.	Moderately ill.	Died 60 hrs. after inoculation; pneumococcus septicemia.
No. 6. Control, washed cocci+immune serum, 0.2 c.c.	Moderately ill.	Died 82 hrs. after inoculation; pneumococcus septicemia.
No. 7. Soaped cocci+immune serum, 0.2 c.c.	Apparently well.	Never noticeably ill. Discarded, well, on May 10, '11.
No. 8. Soaped cocci+immune serum, 0.2 c.c.	Apparently well.	Never noticeably ill. Discarded, well, on May 10, '11.

SERUM INHIBITION.

Blood serum inhibits the bactericidal (and hemolytic) action of soaps. But the effect is not exhausted by this simple observation, for there may be recognized all degrees of inhibition from slight to complete, according to the ratio of quantity of serum to concentration of soap. Further, a given quantity of serum, but one not in excess, inhibits the action of linoleates and linolenates much less than of oleates. For instance, 0.1 cubic centimeter (or 5 per cent.) of serum completely inhibits the action of 0.5 milligram of sodium oleate, yet only slightly retards the dissolving of blood cells and pneumococci by the same quantity of linolenate, and does not quite completely check the action of only one fifth as much.

Hence we were led to inquire into the quantitative relations of serum inhibition and the comparative affinities of the several soaps for serum. Advantage was taken of the already mentioned similarity between the hemolytic and bacteriolytic reactions and a few hemolytic tests were made in order to bring out more clearly slight differences in the degree of lytic action. The next table illustrates the quantitative relations in respect to the inhibition of lysis, existing between serum and the different soaps.

June 6, 1911. Each tube contains 0.3 mg. of soap (dilution of 1 to 10,000); varying quantities of serum; 2 c.c. of a 5 per cent. suspension of dog washed blood cells; with salt solution to a total volume of 3 c.c. The results are expressed in terms of hemolysis after one hour in the incubator and twenty hours in the ice chest.

TABLE II.

Hemolysis.

	1 hour.			20 hours.		
	Sodium oleate.	Sodium linoleate.	Sodium linolenate.	Sodium oleate.	Sodium linoleate.	Sodium linolenate.
1. Control, soap alone.		All comple	te in a few	minutes.		
2. Soap, 0.3 mg.; serum 0.05 c.c.	Great	Complete	Complete	Complete	Complete	Complete
3. Soap, 0.3 mg.; serum 0.1 c.c.	0	Great.	Great.	0	Complete	Complete
4. Soap, 0.3 mg.; serum 0.2 c.c.	0	Moderate.	Great.	0	Complete.	Complete.
5. Soap, 0.3 mg.; serum 0.3 c.c.	0	?	Moderate.	0	Slight	Complete.
6. Soap, 0.3 mg.; serum 0.4 c.c.	0	0	Slight	0	0	Slight.
7. Soap, 0.3 mg.; serum 0.5 c.c.		0			0	0
8. Soap, 0.3 mg.; serum 0.6 c.c.		0			0	0
9. Soap, 0.3 mg.; serum 0.7 c.c.		0			0	0
10. Soap, 0.3 mg.; serum 0.8 c.c.		0			0	0
11. Control, blood cells alone.		0			0	
12. Control, serum 0.4 c.c.		0			0	
13. Control, serum 0.6 c.c.		0			0	
14. Control, serum 0.8 c.c.		0			0	

Thus it is evident that a small quantity of serum only retards, and does not prevent, complete lysis; that to inhibit completely any hemolysis a definite quantity of serum must be present; and that the required quantity is greater for the linoleate than for the oleate, and still greater, in turn, for the linolenate.

Moreover, it has been found that if relatively concentrated solutions of any of these soaps are employed blood cells are dissolved and pneumococci killed in 50 per cent., and even in whole serum. This is shown in the following tables.

June 15, 1911. Horse blood is defibrinated and centrifugalized; the cells being resuspended in portions in 25 per cent., 50 per cent., and whole horse serum. To parts of each suspension a solution of each soap is added so as to make ultimate dilutions of 1 per cent., 0.2 per cent. and 0.1 per cent.

TABLE III.

Hemolysis.

	1 Hour.								
	25 per cent. serum.			50 per cent. serum.			Whole serum.		
	Oleate.	Linoleate.	Linolenate.	Oleate.	Linoleate.	Linolenate.	Oleate.	Linoleate.	Linolenate.
1 per cent. soap	Complete.	Complete.	Complete.	Almost complete.	Complete.	Complete.	Great.	Almost complete.	Complete.
0.2 per cent. soap	Complete.	Complete.	Complete.	Great.	Complete.	Complete.	Slight.	Moderate.	Moderate.
0.1 per cent. soap	Trace.	Complete.	Complete.	0	Trace.	Slight.	0	Trace.	Slight.
	20 Hours.								
1 per cent. soap	Complete.	Complete.	Complete.	Complete.	Complete.	Complete.	Complete.	Complete.	Complete.
0.2 per cent. soap	Complete.	Complete.	Complete.	Almost complete.	Complete.	Complete.	Slight.	Moderate.	Great.
0.1 per cent. soap	Moderate.	Complete.	Complete.	Trace.	Slight.	Moderate.	Trace.	Trace.	Slight.
Control, blood cells alone		0			0			0	

June 17, 1911. Collected the pneumococci in a 24 hours' plain broth culture of No. I. 77 and resuspended portions in 50 per cent. and in whole horse serum to one fourth of the original volume of the culture. Then added solutions of the several soaps to portions of each suspension so as to make ultimate dilutions 1 per cent. and 0.2 per cent. The mixtures were permitted to remain one half hour at room temperature, after which transplantations from each to glucose serum agar were made.

The results are given in terms of growth of pneumococcus after 48 hours' incubation of the transplantations.

TABLE IV.

Bacteriolysis.

	50 per cent. serum.	Whole serum.
1. Control, suspension of cocci alone.	Profuse growth.	Profuse growth.
2. Sodium oleate, 1 per cent.	Sterile.	One colony.
3. Sodium oleate, 0.2 per cent.	Moderate growth.	Less growth than in control.
4. Sodium linoleate, 1 per cent.	Sterile.	Sterile.
5. Sodium linoleate, 0.2 per cent.	Slight growth.	Moderate growth.
6. Sodium linolenate, 1 per cent.	Sterile.	Sterile.
7. Sodium linolenate, 0.2 per cent.	Sterile.	Scant growth.

These observations, as well as the others that relate to the parallelism between the hemolytic or bacteriolytic activities and the quantity of serum required to inhibit them, lead to the belief that the action of the soaps of the unsaturated fatty acids is not due wholly to their power to dissolve lipoids but is due in part to their affinity for protein.

ATTEMPTS TO PREVENT SERUM INHIBITION.

The effort was made to prevent the serum inhibition of the lytic action of the more unsaturated soaps, as in the earlier experiments with sodium oleate. To this end, boric acid was employed first, since it was known to permit oleates to act in the presence of serum. The experiments, in test tubes, were made by adding boric acid in varying quantities to serum, then adding this mixture to the culture of pneumococcus, and then to the whole, sufficient soap solution to make an ultimate dilution within the limit required to produce complete solution of the diplococci in one hour in the absence of serum, *i. e.*, 1 to 4,000 of the linoleate and 1 to 6,000 of the linolenate. Film preparations and transplantations to glucose serum agar

served as indicators. Besides boric acid, a number of salts were tried: sodium borate, silicate, chlorid, citrate, and tartrate. The results were uniformly negative. The pneumococci in such mixtures were neither killed nor dissolved, except in the instance of sodium silicate, where the bacteria were killed, not dissolved, by the antiseptic action of this salt alone.

While these efforts to prevent serum inhibition in the test tube were not successful, it still remained to be seen whether the more unsaturated soaps would exert any influence in pneumococcus infections of the animal body. Experiments were made in which the soaps alone, and in various combinations with normal and immune serum and boric acid, were added to broth cultures and the mixtures injected at once into the peritoneal cavity of rats. Infection was prevented when the concentration of sodium linolenate was 1 to 1,000, a result which agrees with the extremely rapid lytic action of the soaps.

In other experiments, however, where the culture alone was injected first and immediately followed by injections of the soap solutions and the various mixtures of soap, boric acid, and serum, it was only in rare instances that the animals survived. And even then, the experiments being performed in duplicate series, the companion animals died.

In brief, it may be stated that although in the test tube, in the absence of serum, the more unsaturated soaps are much more potent in killing pneumococci, we have been unable so far to obtain with them in the animal body any results which compare at all favorably with those obtained by using sodium oleate. This may be explained by their much greater avidity for protein and their consequent rapid inactivation.

SUMMARY.

In the test tube, sodium linoleate and sodium linolenate kill and dissolve virulent pneumococci more rapidly and in higher dilutions than sodium oleate; otherwise their action is the same. The intensity of action on the cocci is directly proportional to the degree of unsaturation of the acid.

Blood serum inhibits the bacteriolytic and hemolytic action of unsaturated soaps partially or completely, according to definitive

quantitative relations between serum and soap. Small quantities of serum completely inhibit the action of dilute soap solutions, but even whole serum does not prevent bacteriolysis and hemolysis by relatively concentrated soap solutions, *i. e.* 1 per cent. and 0.5 per cent.

A given quantity of serum inhibits the action of the soaps in inverse ratio to their degree of unsaturation.

The hemolytic and bacteriolytic action of soaps of the unsaturated fatty acids is probably due in part to their avidity for protein and not wholly to their ability to dissolve lipoids.

Attempts to prevent serum inhibition of the lytic action of sodium linoleate and sodium linolenate were unsuccessful.

The more unsaturated soaps are not as efficient in preventing fatal pneumococcic infection of animals as sodium oleate.

THE RATE OF REGENERATION OF BLOOD PLATELETS.*

By W. W. DUKE.

(From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins University, Baltimore, Md., and the Pathological Laboratory, University of Kansas Medical School, Kansas City, Kas.)

The experiments to be reported in this paper were performed to determine the rapidity with which blood platelets reappear in the circulation of healthy animals after they have been more or less completely removed by repeatedly withdrawing, defibrinating, and reinjecting blood. Since defibrinated blood is almost free from platelets, the number in the circulation can be reduced to an extremely low level by repeating such a process a number of times at short intervals.

METHODS.

For the purpose of removal of platelets, dogs were anesthetized and, under aseptic technique, were bled from the carotid artery in amounts of 200 to 300 cubic centimeters. This blood was defibrinated by shaking for five minutes with glass beads, strained through gauze, and reinjected slowly into the jugular vein. In experiments I and II, this process was repeated ten and six times respectively, so that at the end of the operation blood obtained by cutting an ear vein showed only a very small clot on standing. In experiments III, IV, and V, the defibrination was less complete.

The animals showed few ill effects following this procedure and except for slight anemia and occasional wound infection, their condition remained good throughout the experiments. During the first few hours after the operation, animals I and II showed a marked tendency to hemorrhage. Bleeding from pricks in the ear veins was so profuse after the obtaining of blood for the platelet counts as to necessitate clamping. There was little bleeding, however, from the

* Received for publication, June 19, 1911.

operation wounds. The latter observation can be accounted for by the fact that the wounds were made before the blood was de-

EXPERIMENT I.

Fox terrier; weight 17 pounds. Ten defibrinations. 200 c.c. of blood withdrawn at each defibrination. Duration of operation 4 hours.

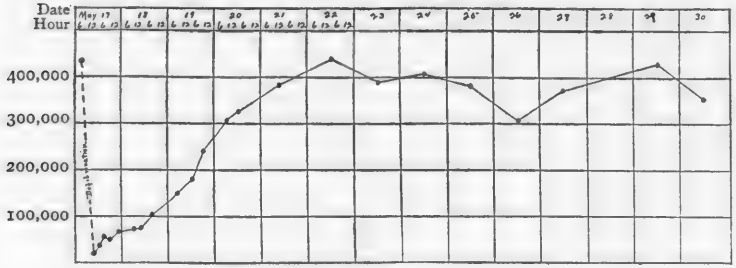


CHART I.

Date.	Platelet count.	Clot.	White count.	Remarks.
May 17				
8.30A.M.	436,000	Firm.	20,000	Hemoglobin 110 per cent. Sahli.
		Blood defibrinated.		
12.15P.M.	21,300	Very slight.	36,000	Bleeds excessively from small cuts in ear.
3 P.M.	38,000	Slight.	38,000	Bleeds excessively from small cuts in ear.
5 P.M.	51,000	Slight.		Bleeds excessively from small cuts in ear.
8 P.M.	43,000	Slight.		
May 18				
1 A.M.	69,000	Not firm.	30,000	Bleeding not markedly prolonged.
10 A.M.	72,000	Firm.	24,700	Bleeding not prolonged.
12 M.	75,000	Firm.	40,000	
6 P.M.	103,000		29,000	
May 19				
8 A.M.	150,000		26,000	Condition of animal good.
3 P.M.	179,000		31,000	
9 P.M.	240,000		40,000	
May 20				
10 A.M.	310,000		26,000	Wound breaking down.
3 P.M.	321,000		21,000	
May 21	383,000		33,000	Wound open.
May 22	444,000		22,400	
May 23	395,000		24,500	Hemoglobin 70 per cent.
May 24	404,000		20,000	Hemoglobin 80 per cent.
May 25	380,000		12,200	Hemoglobin 80 per cent.
May 26	306,000			
May 27	374,000			
May 29	434,000			
May 30	358,000			
May 31	464,000			

fibrinated. The severed vessels therefore became thrombosed while the blood was normal. Animals III, IV, and V, in which the defibrination was not so complete, showed no marked tendency to bleed.

EXPERIMENT II.

Young fox terrier; weight 19.5 pounds. Six defibrinations. 300 c.c. of blood withdrawn at each defibrination. Duration of operation 4 hours.

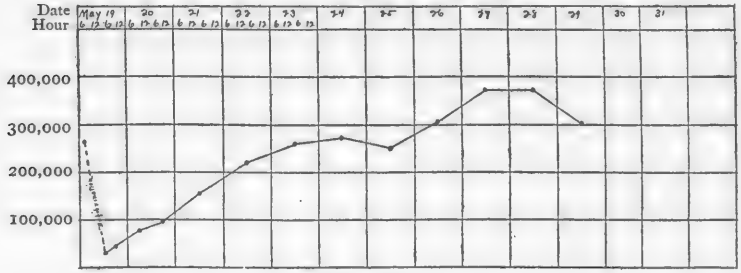


CHART 2.

Date.	Platelet count.	Clot.	White count.	Remarks.
May 19				
9 A.M.	256,000	Firm.	9,900	Hemoglobin 105 per cent. Sahli.
		Blood defibrinated.		
2 P.M.	33,000	Very slight.	21,000	Bleeds excessively from small cuts in ear.
9 P.M.	48,600	None.	26,500	Bleeds excessively from small cuts in ear.
May 20				
10 A.M.	80,000	Soft.	21,000	Hemoglobin 95 per cent.
3 P.M.	97,000	Firm.	31,000	Bleeding from cuts not excessive.
May 21	186,000		9,500	Wound healing. No infection.
May 22	223,000		13,500	
May 23	268,000		9,200	Hemoglobin 75 per cent.
May 24	272,000		8,200	
May 25	258,000			Hemoglobin 70 per cent.
May 26	310,000		20,700	
May 27	375,000		23,000	
May 28	378,000		26,000	
May 29	300,000		23,000	

The platelet counts were made by Wright and Kinnicutt's method.[1] In this method the blood is diluted in a blood pipette (1 to 100 dilution) with a mixture of two parts of a solution of brilliant cresyl blue (aqueous solution 1 to 300) and three parts of a solution of potas-

[1] Wright and Kinnicutt, *Jour. Am. Med. Assn.*, 1911, lvi, 1457.

sium cyanid (aqueous solution 1 to 1,400). This mixture must be filtered and used immediately. The exact proportion of stain and cyanid used in the mixture is of the greatest importance, and in studying blood of different species of animals the proportion must be varied somewhat. If the amount of cyanid is excessive, both the

EXPERIMENT III.

Fox terrier; weight 21 pounds. Three defibrinations. 250 c.c. of blood removed at each defibrination. Duration of operation 1 hour.

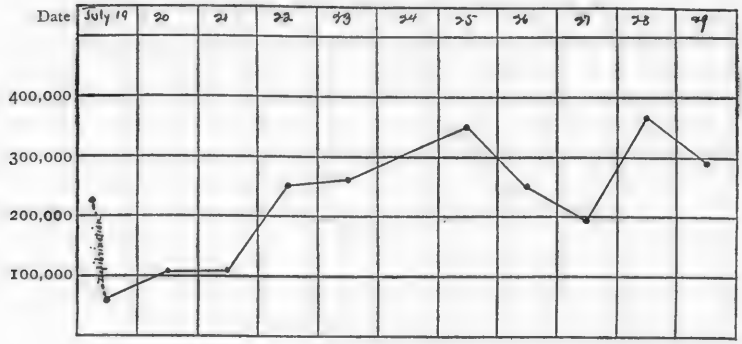


CHART 3.

Date.	Platelet count.	Clot.	Remarks.
July 19	225,000	Firm.	Blood defibrinated.
July 19	84,000	Soft.	Bleeding from small cuts in ear slightly prolonged.
July 20	108,000	Firm.	Bleeding not excessive.
July 21	110,000		Wound breaking down.
July 22	254,000		
July 23	264,000		
July 25	352,000		
July 26	249,000		
July 27	192,000		
July 28	370,000		
July 29	290,000		

platelets and leucocytes disintegrate, while if the amount is deficient the red cells are not completely laked and the platelets clump. Requisites for a good mixture are that the red cells must be laked, the protoplasm of the leucocytes well stained, and the platelets evenly distributed and present a blue or violet color. For dog

blood, a mixture of two parts of stain to five parts of cyanid solution gives the best results. The platelets are counted in a .100

EXPERIMENT IV.

Fox terrier; weight 12 pounds. One defibrination. 200 c.c. of blood removed.

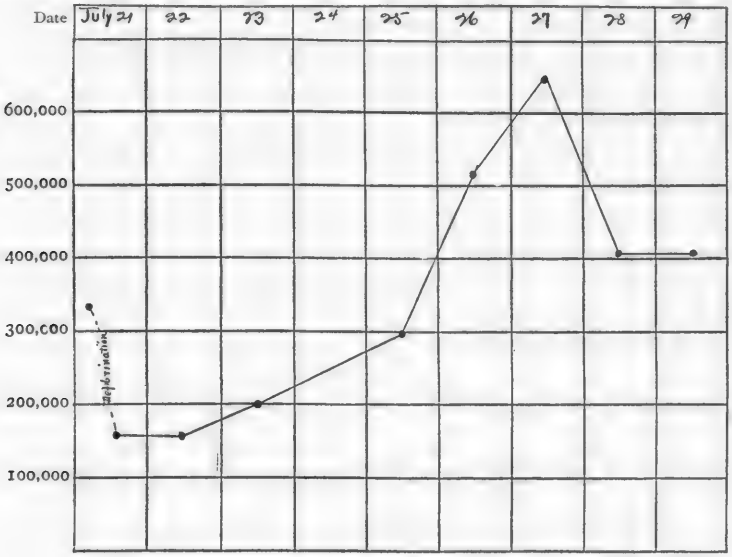


CHART 4.

Date.	Platelet count.	Remarks.
July 21	328,000	Hemoglobin 105 per cent. Sahli.
		Blood defibrinated.
July 21	161,000	
July 22	158,000	Wound open.
July 23	200,000	
July 25	300,000	Animal not lively. Losing weight.
July 26	520,000	Slight infection of ear where pricked.
July 27	644,000	Animal seems sick.
July 28	412,000	Hemoglobin 65 per cent.
July 29	404,000	Hemoglobin 70 per cent.

millimeter Zeiss chamber with a high dry lens. It is hardly necessary in this paper to discuss the relative merits of the various methods for counting platelets. It may be said simply that if the

technique is carried out quickly and if the blood used for the determination is flowing freely, constant results can be obtained by this method.

The platelet count in different dogs varies considerably. In carrying out experiments in another connection, we have noted

EXPERIMENT V.

Fox terrier; weight 12 pounds. Two defibrinations. 300 c.c. of blood removed at each defibrination.

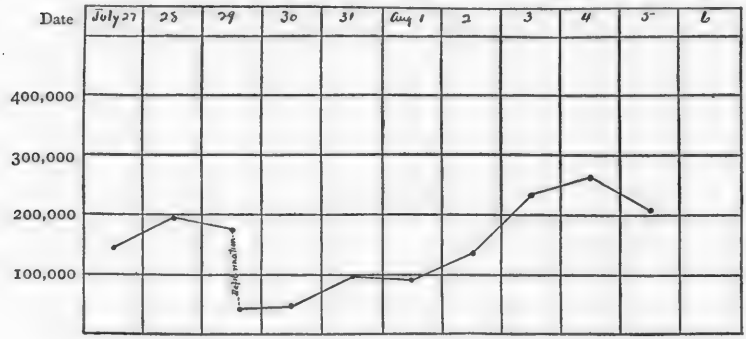


CHART 5.

Date.	Platelet count.	Remarks.
July 27	140,000	
July 28	194,000	
July 29	177,000	Hemoglobin 100 per cent. Sahli.
		Blood defibrinated.
July 29	39,200	Hemoglobin 95 per cent.
July 30	48,300	Hemoglobin 85 per cent.
July 31	92,000	Hemoglobin 80 per cent.
Aug. 1	88,000	Wound breaking down. Animal looks rather quiet and sick.
Aug. 2	135,000	
Aug. 3	236,000	
Aug. 5	263,000	
Aug. 8	204,000	

counts as high as 800,000 and as low as 180,000. In an individual dog, however, under normal conditions the count taken from day to day rarely varies as much as 100,000.

In the first two experiments, in order to detect slight changes, four counts were made at each determination; in the last three experiments, two. The average results are recorded in the charts.

DISCUSSION.

An examination of the data shows that the reappearance of platelets in the circulation after their removal by repeated defibrination of blood is remarkably rapid. In each of the five experiments, the normal count was reached in from three to five days. This was true whether the removal of platelets had been almost complete (78 to 95 per cent.) or only partial (50 to 66 per cent.), whether the normal count for the animal was high (436,000) or low (180,000). The rate of reappearance was in some instances highly remarkable and amounted to as much as half the normal number of platelets in the blood in one day.

A rather striking difference can be seen between the first two and the last three experiments. In the first two, the rise in the platelet count began immediately after the operation and proceeded quite steadily to normal or a little above. In the last three, however, there was little change in the count until the second or third day, and then followed a rapid increase which in one case (experiment IV) rose high above the normal. It may be pointed out that in the experiments in which the rise appeared to be delayed, the platelets had been less completely removed from the circulation. Whether this delay was due to a less intense demand for platelet regeneration or to injury and premature disintegration of the platelets remaining in the circulation after the operation is a question which cannot be definitely decided, and it hardly warrants discussion.

The experiments were carried out mainly in the hope of throwing light on the rate of generation of platelets which obtains under physiologic conditions.

It is conceivable that after the removal of platelets from the blood by defibrination, the generation is continued at the usual rate and that the curves in experiments I and II (experiments in which the removal of platelets was almost complete) give an approximate picture of the normal rate of platelet formation in dogs. In view of our ignorance, however, as to whether or not a lack of platelets is a stimulus to platelet generation, such a conclusion is not justifiable. In fact, the existence of such a stimulus would seem most probable

and one would be almost safe in assuming that the rate of generation in the experimental condition was abnormally rapid.

The experiments show, however, that under conditions not markedly pathologic, the generation of platelets is extremely rapid and, in comparison with that of the red cells under a somewhat analogous state of stimulation (severe anemia[2]), is much more rapid. It may be added in this connection that fluctuations in the platelet count in human beings under both normal and pathologic conditions are greater and more rapid proportionally than those of the red count. In a case of tuberculosis,[3] for example, it was observed that just preceding an attack of purpura hemorrhagica, the platelet count fell from 210,000 to 4,000 within a period of three days. After remaining at this level for five days it suddenly began to rise at an average rate of 100,000 a day until a count of 720,000 was reached. The red count under no circumstances shows such marked and rapid changes. It has been noted also[4] that the platelets of normal individuals introduced into three patients having hemorrhagic disease of the type just mentioned disappeared within a period of about three days. The red cells introduced at the same time gave no evidence of rapid disintegration.

It is evident that under certain conditions the disappearance of platelets may be extremely rapid, and that under the influence of certain demands the rate of generation also may be extremely rapid. It is hard to believe that such rapid regeneration could take place if the demand for it was a rare one, and a view that rapid disintegration and replacement of platelets is a more or less physiological occurrence would seem to fit well with the data. It is possibly justifiable, therefore, to suggest that platelets under the usual conditions of life are rapidly disappearing either through utilization or disintegration and that the count is kept constant under a given set of conditions by as rapid a rate of formation. In comparison with the erythrocytes, both the rate of destruction and rate of genera-

[2] P. M. Dawson, Effects of Venous Haemorrhage and Intravenous Infusion in Dogs, *Am. Jour. Physiol.*, 1900, iv, 1.

[3] A complete report of this case is to appear in *Deutsch. Arch. f. klin. Med.*, 1911.

[4] W. W. Duke, The Relation of Blood Platelets to Hæmorrhagic Disease *Jour. Am. Med. Assn.*, 1910, lv, 1185.

tion would seem to be proportionately more rapid. If such is the case, the life cycle of the platelet must be considered extremely short and can possibly be reckoned in days.

CONCLUSIONS.

By repeatedly withdrawing, defibrinating, and reinjecting blood, the platelet count in dogs can be reduced to an extremely low level.

When the reduction in platelets and fibrinogen has been carried to an extreme degree the animals display a marked tendency to bleed.

The regeneration of platelets after their removal by defibrination is remarkably rapid and amounts, on an average, to about one fifth of the entire number in the blood per day.

From the experiments and from observations on the platelet count in human beings, it is believed that under the usual conditions of life platelets are utilized or destroyed and replaced in enormous numbers daily, and that the life cycle of the platelet is extremely short,—possibly a matter of a few days.

My sincere thanks are due to Dr. G. H. Whipple, of Johns Hopkins Medical School, and Professor M. A. Barber, of the University of Kansas Medical School, in whose laboratories the experiments were carried out, and also to Drs. J. H. Wright and Roger Kinnicutt for their excellent method of counting blood platelets.

THE EXCRETION OF DEXTROSE IN THE STOMACH AND THE SMALL INTESTINE.*

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INTRODUCTION.

The question of the elimination of dextrose from the blood into the gastro-intestinal canal has become a subject of experimental investigation and developed in connection with the recent studies on the production of glycosuria by the intravenous injection of solutions of sodium chlorid. The following is a brief review of these studies, some of the results of which gave rise to the investigations which are to be reported in this paper.

About four decades ago, Bock and Hoffmann[1] observed that intravascular injections of sodium chlorid are followed by glycosuria, an observation soon confirmed by Külz.[2] Recently the subject was taken up again by two American investigators, M. H. Fischer[3] and O. H. Brown.[4] While these authors carried out their work at about the same time, but independently of one another and in different laboratories (Fischer working in Loeb's laboratory at the University of California, and Brown in the Physiological Laboratory of the University of Chicago), both were nevertheless stimulated to their investigations by the studies and conclusions of J. Loeb; namely, that sodium salts increase and calcium salts decrease the irritability of nerve and muscle. Fischer found that when relatively large quantities of an M/6 solution of sodium chlorid are injected rapidly into the veins of rabbits the first effect is a greatly increased diuresis, which is followed later by the appearance in the urine of dextrose that may persist for many hours. The appearance of glycosuria, however, can be prevented by the addition of calcium chlorid. An intra-arterial injection of sodium chlorid in the direction of the medulla oblongata causes also, according to Fischer, the appearance of glycosuria which cannot be suppressed by the addition of calcium chlorid. Fischer assumes that the diuresis is produced in

* Received for publication, June 22, 1911.

[1] Bock and Hoffmann, *Arch. f. Anat. u. Physiol.*, 1871, 550.

[2] Külz, *Beiträge zur. Anat. u. Physiol.*, von Eckard, 1872, vi, 117.

[3] M. H. Fischer, University of California Publications, Physiology, 1902–1903, i, 77 and 87; *Arch. f. d. ges. Physiol.*, 1905, cvi, 80; *ibid.*, 1905, cix, 1.

[4] O. H. Brown, *Am. Jour. Physiol.*, 1904, x, 378.

both instances by a stimulation of the kidney cells by sodium chlorid, while the glycosuria is caused by a stimulation of the "diabetic centre" in the medulla.

Underhill and Closson,[5] however, arrived at other conclusions. They analyzed the blood for its content of dextrose during the period of glycosuria produced by the intravascular injection of sodium chlorid and found the result to differ with the mode of injection. After intra-arterial injection of the sodium chlorid a hyperglycemia is produced, and after intravenous injections, a hypoglycemia. In the latter case, an injection of calcium salts causes a return of the dextrose to normal, or even an increase above normal, in the blood. Underhill and Closson assume that the glycosuria after intravenous injection of sodium chlorid presents a condition of "renal diabetes," or one in which the sodium chlorid causes an increase in permeability of the kidney epithelia for sugar with a consequent hypoglycemia; whereas the calcium salts tend to diminish the permeability. On the other hand, that which is caused by the intra-arterial injection of sodium chlorid in the direction of the medulla is brought about by asphyxia, because it interferes, according to these authors, with the activity of the respiratory centre.

McGuigan and Brooks[6] believe that the sugar in the blood is not free, but exists in combination with a protein molecule, and they assume that sodium chlorid and other salts, which cause experimental glycosuria, accomplish it by freeing the dextrose from its combination with the protein molecule; calcium salts, on the other hand, preventing such a liberation of the dextrose. In some of their experiments, solutions of calcium chlorid, when injected into the blood, did not prevent the occurrence of glycosuria, due to subsequent dextrose infusion. The authors see in this effect a proof that calcium does not decrease the permeability of the kidney epithelia to sugar, as was assumed by Underhill and Closson. Underhill and the present writer,[7] however, showed later that the glycosuria in the experiments of McGuigan and Brooks was produced by the action of the strong solutions of the calcium salt upon the respiratory centre, thereby causing respiratory insufficiency and asphyxia. Furthermore, the assumption of Underhill and Closson of the diminution by calcium of the permeability of kidney epithelia had no reference to epithelia of normal kidneys but only to epithelia, the permeability of which had been previously increased by the action of sodium chlorid.

J. B. MacCallum,[8] who also worked in Loeb's laboratory, studied the influence of the intravenous injection of solutions of sodium chlorid upon the elimination of fluid and sugar into the stomach and intestine of rabbits. He found, under these conditions, an increase of secretion of fluid into the stomach and small intestine and that both viscera contained sugar. The quantities of the eliminated fluid and of sugar in the stomach and intestine were increased if the kidneys were removed previous to the infusion of the salt solution. MacCallum speaks of "intestinal polyuria" and "intestinal diabetes," and compares

[5] Underhill and Closson, *Am. Jour. Physiol.*, 1906, xv, 321.

[6] McGuigan and Brooks, *Am. Jour. Physiol.*, 1907, xviii, 256.

[7] Underhill and Kleiner, *Jour. Biol. Chem.*, 1908, iv, 395.

[8] J. B. MacCallum, University of California Publications, Physiology, 1904, i, 125.

the effects upon the stomach and intestine with the effects which the intravenous injections of sodium chlorid exert upon the kidneys. "It seems," he says, "that the infusion of M/6 NaCl solution causes sugar to appear in the blood. On reaching a certain concentration, it is secreted by the kidneys. If the kidneys are cut off from the circulation, the sugar is excreted by the intestine." He looks upon the intestine as a supplementary excretory organ. MacCallum seems to have made altogether only four experiments, and only in one were the kidneys not removed.

While discussing the results and views of MacCallum, Fischer and Moore[9] point out first that the quantity and the concentration of the sodium chlorid solution employed by him were not sufficient to bring on any glycosuria. Then they show by experiment that hyperglycemia alone, produced by the diabetic puncture, injection of morphin, or intravenous injections of dextrose or cane sugar, does not cause an elimination of sugar in the intestine or stomach. However, hyperglycemia having been caused by these injections, an intravenous injection of sodium chlorid, even in quantities and concentrations which alone are insufficient to produce glycosuria, will cause an excretion of dextrose into the small intestine and possibly into the stomach also. Fischer and Moore explain the mechanism of the process on the assumption that sodium chlorid increases the permeability of the intestinal epithelia and thus permits the escape of dextrose from the blood, when it is present there in excess. The findings of MacCallum are explained by Fischer and Moore by the fact that in his experiments the rabbits received 5 c.c. of a 1 per cent. morphin solution, a dose sufficient to produce hyperglycemia, after which the sodium chlorid injection rendered the intestine permeable to this excess of sugar. Fischer and Moore state that the small intestine and stomach of normal rabbits are usually free from dextrose. We may add that they still speak of "hyperglycemia" caused by intravenous injections of sodium chlorid, although there is no evidence in their paper that they reëxamined this point experimentally. As far as we can see, the statement of Underhill and Closson, that when sodium chlorid has been injected intravenously the glycosuria is accompanied by a hypoglycemia, has not yet been controverted by any experimental investigation.

As regards the question of elimination of dextrose into the stomach and intestine,—the question which concerns us particularly in this investigation,—we find in the first place that Fischer and Moore believe that no such elimination takes place under normal conditions. MacCallum's findings seem to agree with this view. Furthermore, MacCallum believes that an elimination of dextrose in the stomach and intestine takes place whenever hyperglycemia exists, and the "intestinal diabetes" after an intravenous injection of sodium chlorid is simply one instance of this general rule. Fischer and Moore, on the other hand, believe that hyperglycemia alone does not lead to an excretion of dextrose in the alimentary

[9] Fischer and Moore, *Am. Jour. Physiol.*, 1907, xix, 314.

canal. According to them, this occurs only when, in addition to the hyperglycemia, the intestinal epithelia are rendered more permeable, which is the case when sodium chlorid is injected intravenously. Finally, there is the statement of MacCallum, that after removal of the kidneys a greater excretion of dextrose in the intestinal canal takes place, a phenomenon to be interpreted, according to him, as brought about by vicarious activity of the intestine.

On the basis of the foregoing considerations, I carried out several series of experiments to obtain answers to the following questions:

(1) Do the stomach and the small intestine of rabbits normally contain dextrose, or a reducing substance, in a demonstrable amount? (2) Will an intravenous injection of dextrose, sufficient to produce glycosuria, cause an elimination or an increase of the elimination of dextrose into the stomach and intestine? (3) Will, in the latter case, a double nephrectomy cause an increase of excretion of dextrose in the gut, and will this increase be, in comparison with the elimination through the kidneys, sufficiently large to constitute an instance of vicarious activity?

EXPERIMENTAL RESULTS.

METHODS.

The experiments were performed on rabbits which, in almost all cases, had been fed for several days previously on hay and cabbage; that is, on a diet poor in easily convertible carbohydrates. The analysis for dextrose in the alimentary canal was confined to the contents of the stomach and the small intestine. The contents of the latter were obtained by tying cannulas in the upper part of the duodenum and at the lower end of the ileum; by means of a pressure bottle, the entire small intestine was irrigated *in situ* with about a liter of 0.9 per cent. sodium chlorid. This was done immediately after death. The procedure in all cases was as follows: the animal was etherized, the abdomen opened, the end of the small intestine ligated and then the trachea clamped. Immediately after the last sign of life had passed away, the cannulas were inserted and the irrigation started. The ligation of the intestine was done

to prevent the removal of its contents into the cecum by the violent peristalsis which sweeps over the intestines during the period of asphyxia. Urine was collected by means of a cannula tied into the neck of the bladder. Solutions of dextrose were administered intravenously from a burette connected to a cannula, tied in the left external jugular vein. The kidneys were exposed through the lumbar region and were eliminated by ligating their blood-vessels. All operative procedures were carried out under ether anesthesia, except in a few instances in which a cannula was inserted into a jugular vein under local cocaine anesthesia.

For the determination of dextrose (or rather reducing substances) in the small intestine, the following procedures were employed. The irrigated fluid was acidified with acetic acid, filtered, and concentrated. The concentrated fluid was then either filtered and analyzed or was treated with phosphotungstic acid, filtered, neutralized, and then analyzed. The analysis was made usually by the Pavy-Vernon[10] method. However, in four experiments in which the fluid was treated according to the first mentioned procedure, some analyses were also made by the Allihn method. The following short table shows that the results obtained by the two methods do not differ greatly.

Number of Experiment.	Pavy-Vernon.	Allihn.
55.	0.16 gm.	0.15 gm.
56.	0.17 gm.	0.14 gm.
66.	0.07 gm.	0.04 gm.
70.	0.24 gm.	0.24 gm. 0.23 gm.

The stomach contents were macerated with water, strained through cotton gauze and centrifugalized. In some cases, the fluid was merely boiled and then analyzed; however, better results were obtained by treating it with phosphotungstic acid, filtering and neutralizing before subjecting it to the analysis. The determination of the reducing substances was made by the Pavy-Vernon method; determinations by the Allihn method were here unsatisfactory.

[10] Vernon, *Jour. Physiol.*, 1902, xxviii, 156.

No claim is made and can be made for extreme accuracy of the results, on account of the presence of interfering substances in both the intestinal and gastric contents. Whenever the qualitative test was doubtful it is indicated in the tables by an interrogation mark.

The urines were always analyzed by the Pavy-Vernon method.

THE PRESENCE OF DEXTROSE IN THE STOMACH AND SMALL INTESTINE OF NORMAL RABBITS.

In the four experiments of MacCallum's on the small intestine, it is mentioned in the protocols that the fluid obtained before the infusion of sodium chlorid was started contained no sugar. Regarding the stomach, MacCallum says categorically that in normal rabbits it contains no sugar, although his experience apparently included only one or two experiments.

Fischer and Moore studied the subject in seven rabbits. Sugar was absent from the small intestine in every case. Regarding the stomach they state that it contains sugar in proportion to the character of the food. When the rabbits were fed on cabbage or the tops of carrots there was very little or no sugar; when they were fed with the pulp of carrots sugar was present.

Our experiments were performed on rabbits fed with cabbage and hay. In a series of six normal animals, the contents of the small intestine and of the stomach were analyzed for dextrose. No protocol of an experiment will be given. The following table (table I) presents the results.

TABLE I.

Normal Rabbits. No Dextrose Injected.

Number of experiment.	Weight in kilos.	Dextrose in small intestine in gm.	Dextrose in stomach in gm.
63	1.29	0.02(?)	0.07–0.12(?)
64	1.25	0.10	0.12
65	1.57	0.04	0.07–0.14
66	1.38	0.07	0.10
67	1.97	0.04–0.07(?)	0.15–0.18
69	1.67	0.03(?)	0.04
Average		0.04	0.09

As the table shows, our results with the small intestine differ from those of MacCallum and of Fischer and Moore. We found a measurable, though variable, quantity of dextrose present in most of the experiments. Fischer and Moore state expressly that "no reduction is obtained in the intestinal wash water even after pro-

longed boiling with a Fehling's solution." With regard to the stomach contents, our findings are in better agreement with those of Fischer and Moore, although with such a diet as was used in our experiments Fischer and Moore state that there is little or no sugar present. We wish to call attention to the fact that in our experiments the amount of dextrose in the contents of the stomach was in every case definitely larger than in the small intestine.

In three experiments the contents of the stomach and of the small intestine of nephrectomized rabbits, which otherwise received no treatment, were examined for the presence of dextrose. Table II shows the result.

TABLE II.

Nephrectomized Rabbits—No Dextrose Injected. Operated on the Preceding Day.

Number of experiment.	Weight in kilos.	Dextrose in small intestine in gm.	Dextrose in stomach in gm.
83	1.61	0.02(?)	0.04(?)
85	1.23	0.02(?)	0.06
86	1.54	0.02(?)	0.03(?)

The result may seem somewhat surprising. There was perhaps no good reason to expect a greater content of dextrose in the stomach and intestine in nephrectomized rabbits, since normally the kidneys do not excrete sugar. But why should there be definitely less? However, all three animals were operated on the previous day, and we may assume that because they had taken no food or drink the blood of the rabbits contained less fluid as well as less dextrose; hence the diminished dextrose content of the excretion of the intestines.

THE ELIMINATION OF DEXTROSE INTO THE STOMACH AND SMALL INTESTINE AFTER AN INTRAVENOUS INFUSION OF DEXTROSE.

As stated above, MacCallum assumed that any hyperglycemia leads to an elimination of dextrose into the stomach and small intestine. Fischer and Moore, however, insist that the mere increase of sugar in the blood does not cause an excretion of sugar into the gastro-intestinal canal. In their experiments with morphin and diabetic puncture, which led to glycosuria, no sugar appeared in the

stomach and intestine. Neither was there any sugar found in an experiment in which dextrose was injected intravenously leading to glycosuria. In this experiment, 124.8 cubic centimeters of a one fourth molecular solution of dextrose (4.5 per cent.), *i. e.*, 5.6 grams, were injected intravenously in the course of one hour. The sugar eliminated in the urine amounted to 0.62 gram, that is, only about 11 per cent. of the injected dextrose. The volume of the urine amounted to only twenty-five cubic centimeters and the percentage of the dextrose in it was 2.48 per cent. Fischer and Moore do not state the exact weight of the animal.

In view of the fact that Fischer and Moore made only one experiment of this kind and with a quantity of dextrose which did not lead to a strong glycosuria, we studied this question in a larger series of animals. In our experiments, the warm solution of dextrose was run, as stated, from a burette into one of the external jugular veins. At the end of the infusion, the animal was permitted to live fifteen minutes before being killed and the contents of the viscera were collected in the manner described above. During the experiment, the animal was kept warm by means of an electric thermal pad. The urine was collected throughout the experiment from a cannula in the bladder.

Normal Rabbits.—The experiments were carried out in two series of six rabbits each. In the first series, four grams of dextrose per kilo. of body weight were administered. In order that the same volume of fluid per kilo. should be used in both this and the next series, the concentration of the dextrose solution in this series was made 11.4 per cent. The following is an abbreviated protocol of an experiment.

Experiment 60.—Female rabbit, 1,470 grams.

Animal placed on an electric thermal pad. Under ether anesthesia, cannulas were introduced into the left jugular vein and into the neck of the bladder.

2:05 P. M. Operation finished.

2:22 P. M. Intravenous infusion of warm 11.4 per cent. dextrose solution started.

2:34 P. M. Urine starts to flow after 18 c.c. of solution have run in.

2:52 P. M. Dextrose infusion finished. Total solution injected, 51.5 c.c. = 5.87 gm. dextrose.

3:07 P. M. Etherized. Abdomen opened and ileum tied near cecum; trachea

(and esophagus) clamped. Soon after death, cannulas were introduced in the upper part of the duodenum and the end of ileum, and the small intestine was irrigated with 900 c.c. 0.9 per cent. sodium chlorid; stomach contents extracted with water. These fluids and the urine were now analyzed as described above. In the urine (39 c.c.) was found 1.62 gm.; in the contents of small intestine, 0.07 gm.; and in that of the stomach, 0.06 gm. of dextrose (or reducing substance); respectively: 27.6 per cent., 1.2 per cent. and 1.0 per cent. of the amount introduced.

The following table (table III) contains the results in detail of these six rabbits.

TABLE III.

Normal Rabbits. Four Grams of Dextrose per Kilo. of Body Weight Injected Intravenously.

Number of experiment.	Weight of animal in kilos.	Duration of dextrose injection in minutes.	Dextrose in urine.			Volume of urine in c.c.	Dextrose in small intestine.		Dextrose in stomach.	
			In grams.	Percentage of amount injected.	As dextrose solution. Percent.		In grams.	Percentage of amount injected.	In grams.	Percentage of amount injected.
59	2.66	41	4.08	38.3	4.0	102	0.15	1.4	0.23	2.2
60	1.47	30	1.62	27.6	4.2	39	0.07	1.2	0.06	1.0
61	0.95	20	0.63	16.2	3.3	19	0.07	1.8	0.01?	0.3?
62	0.80	17	0.50	15.6	4.6	10.8	0.06	1.9	0.00	0.0
74	1.69	23	1.52	22.6	4.8	32	0.20	3.0	0.06	0.9
75	1.47	33	1.55	26.4	7.4	21	0.11	1.9	0.13	2.2
Average			1.65	24.5	4.4	37	0.11	1.9	0.08	1.1

As Fischer and Moore state, in regard to the weight of their animal (experiment 5), that it was "medium sized," only an approximate comparison with any of our experiments can be made. The rabbit of our experiment 60, which may be considered medium sized, received about the same quantity of dextrose as Fischer and Moore's rabbit. We may therefore compare the results of these two experiments. Fischer and Moore injected 5.6 grams (as 4.5 per cent.) in one hour and obtained twenty-five cubic centimeters of urine containing 2.5 per cent. dextrose, *i. e.,* 11 per cent. of the amount injected. We gave 5.9 grams (as 11.4 per cent.) in a half hour and obtained thirty-nine cubic centimeters of urine containing 4.2 per cent. dextrose, equivalent to 27.6 per cent. of the quantity injected. It is probable that this striking difference was due to the greater concentration of our solution, as well as to the greater rapidity with which this solution was injected. The average amount of

the dextrose eliminated into the small intestine in this series of experiments was slightly larger than that of the first series of normal rabbits in which no dextrose was injected. There is no perceptible difference in the amount of dextrose in the contents of the stomach of both series.

In another series of six rabbits, seven grams of dextrose per kilo. body weight were injected intravenously, in 20 per cent. concentration. The following protocol will serve as an illustration.

Experiment 56.—Gray and black female rabbit, 2,180 grams.
Etherized and operated as in experiment 60.
2:15 P. M. Operation finished.
2:27 P. M. Infusion of 20 per cent. dextrose into the jugular vein begun.
2:29 P. M. Urine starts to flow after 7 c.c. of solution have been injected.
3:04 P. M. Dextrose infusion finished. Total solution injected 76.5 c.c. = 15.3 gm. dextrose.
3:21 P. M. Etherized and proceeded as in experiment 60.
The urine (131 c.c.) contained 6.55 gm. of dextrose; the small intestine, 0.17 gm.; and the stomach, 0.27 gm.; equivalent respectively to 42.8 per cent., 1.1 per cent. and 1.8 per cent. of the amount of dextrose injected.

The details of the results of this series of experiments are presented in table IV.

TABLE IV.

Normal Rabbits. Seven Grams of Dextrose per Kilo. of Body Weight Injected Intravenously.

Number of experiment.	Weight of animal in kilos.	Duration of dextrose injection in minutes.	Dextrose in urine.			Volume of urine in c.c.	Dextrose in small intestine.		Dextrose in stomach.	
			In grams.	Percentage of amount injected.	As dextrose solution. Percent.		In grams.	Percentage of amount injected.	In grams.	Percentage of amount injected.
52	1.84	32	7.45	57.3	5.4	138	0.14	1.1	0.04(?)	0.3(?)
55	1.53	28	3.53	33.0	3.8	93	0.16	1.5	0.04	0.4
56	2.18	37	6.55	42.8	5.0	131	0.17	1.1	0.27	1.8
72	1.38	26	3.04	31.3	4.4	69	0.09	0.9	0.07	0.7
73	1.69	35	5.68	47.7	4.5	125	0.06	0.5	0.04	0.3
105	1.72	43	4.00	33.3	4.6	87	0.26	2.2	0.18	1.5
Average			5.04	40.9	4.7	107	0.15	1.2	0.10	0.8

The percentage of dextrose eliminated in the urine is, in this series, definitely larger than in the last, in which only four grams of dextrose per kilo. of body weight were given. The elimination of dextrose into the small intestine is definitely larger than that of the

animals in either of the previous series of experiments, although even these quantities seem to be very small when compared with those eliminated through the kidneys. The elimination into the stomach is, in this series, also slightly increased above that of the other series. However, the increase in the elimination into the stomach and intestine is proportionately far below the increase in the quantity of the injected dextrose. The percentages of elimination into the stomach and intestine are therefore smaller in the series with seven than in the series with four grams per kilo. of body weight.

INTRAVENOUS INJECTION OF DEXTROSE INTO NEPHRECTOMIZED RABBITS.

In a final series of experiments on six rabbits double nephrectomy was performed and dextrose subsequently injected intravenously in a dosage of seven grams per kilo. of body weight. In two cases, the sugar was injected soon after the nephrectomy; in the remaining experiments the infusion was made on the next day. The following protocol is an example.

Experiment 80.—Light brown female rabbit, 1,740 grams.

January 31, P. M. Under ether anesthesia, both kidneys ligatured.

February 1. Under local cocaine anesthesia a cannula was introduced into the jugular vein. Animal placed on electric pad.

1:52 P. M. Intravenous injection of warm 20 per cent. dextrose solution. Animal hyperesthetic.

2:47 P. M. Dextrose injection ended. Total infusion 61 c.c. = 12.2 grams dextrose.

3:02 P. M. Etherized, etc., as described in previous experiments. The small intestine contained reducing substances amounting to 0.27 gram, equal to 2.2 per cent. of the amount of dextrose injected; the stomach contained 0.16 gram or 1.3 per cent. of the total dextrose injected.

Table V shows the details of this last series of experiments.

The amount of dextrose in the stomach and intestine is on the average slightly greater in the nephrectomized animals than in the normal animals which received also seven grams per kilo. of body weight in 20 per cent. concentration. The increase, however, is obviously insignificant, considering especially that the nephrectomized rabbits retain the 40 per cent. and more of the injected dex-

TABLE V.

Nephrectomized Rabbits. Seven Grams of Dextrose per Kilo of Body Weight Injected Intravenously.

Number of experiment.	Weight of animal in kilos.	Duration of dextrose injection in minutes.	Dextrose in small intestine.		Dextrose in stomach.		Remarks.
			In grams.	Percentage of amount injected.	In grams.	Percentage of amount injected.	
70	1.61	47	0.24	2.1	0.24	2.1	Operated on preceding day
78	1.65	47	0.09	0.8	0.13	1.1	Operated on preceding day
79	1.83	37	0.27	2.1	0.09	0.7	Operated on preceding day
80	1.74	55	0.27	2.2	0.16	1.3	Operated on preceding day
81	1.43	37	0.27	2.7	0.09	0.9	Operated on same day
82	1.33	35	0.30	3.2	0.16	1.7	Operated on same day
Average			0.24	2.2	0.15	1.3	

trose which was eliminated through the intact kidneys of the normal rabbits.

DISCUSSION.

In a series of normal rabbits in which no dextrose was injected, the small intestine of some contained definitely measurable quantities of dextrose. Neither MacCallum nor Fischer and Moore found any traces of sugar in the small intestine. The latter writers say: "That traces of sugar could be found with more delicate analytical methods is not disputed." But a quantity of 0.1 gram (experiment 64) is surely more than such a trace and is even more than the quantity found by Fischer and Moore in one of their two experiments in which an intravenous injection of dextrose and sodium chlorid was given. We are sure that in our experiments the presence of dextrose was not due to an admixture of blood; this was carefully avoided. Besides, 0.1 gram is probably all the sugar which the entire blood of this animal contained. Neither is it probable that the sugar was simply a remnant of the food; the animals were kept on a diet poorer in readily convertible carbohydrates than the animals of Fischer and Moore. It is also not very probable that the presence of dextrose in our cases was due to an acute hyperglycemia produced by the strangulation of the animal. We are, nevertheless, prepared to state only the fact that in some of our experiments the small intestine of normal animals contained immediately after death measurable quantities of a reducing substance,

preferring not yet to draw the definite conclusion, that in living animals dextrose is eliminated normally into the intestine.

What we said of the intestine applies also to the stomach; here the amounts of dextrose were even larger than those found in the intestine. Our actual findings do not differ materially from those of Fischer and Moore, although they found dextrose in the stomach only when the animals were fed on a diet rich in easily convertible carbohydrates (carrot pulp). MacCallum's opposite statement was based on too few experiments.

The experiments with the intravenous injections of dextrose seem to settle definitely the fact that a certain degree of hyperglycemia will undoubtedly cause an excretion, or, perhaps better, an increase of excretion of dextrose into the intestine and the stomach. In the series of experiments in which seven grams of dextrose per kilo. of body weight were injected in a 20 per cent. solution, the quantities of dextrose in the small intestine were unmistakably larger than those present in the small intestine of the rabbits which received only four grams of dextrose per kilo. of body weight, injected in a concentration of 11.4 per cent. The duration of the injection did not differ materially in either series of experiments. It is therefore evident that the intravenous injection of a larger quantity of dextrose in about the same unit of time causes undoubtedly a greater hyperglycemia for a certain length of time. It is interesting to note that in the series of experiments with seven grams of dextrose per kilo. of body weight, the volume of urine and the amount of sugar eliminated in it were considerably greater than in the series of experiments with only four grams per kilo. of body weight. Evidently the injection of a greater quantity of dextrose in the same unit of time causes also a greater polyuria and glycosuria. In our experiments, the greater elimination of dextrose in the intestine and the stomach was due to the injection of dextrose alone; and since no additional injection of sodium chlorid was made, we need not discuss the possibility of the production of a greater permeability of the intestinal epithelia being a factor in the elimination of dextrose in the gastro-intestinal canal. Fischer and Moore report only one experiment with dextrose. In this experiment, the amount of dextrose injected in a unit of time was much smaller than in our experi-

ments. The failure of these authors to obtain an elimination of dextrose into the gastro-intestinal canal when a hyperglycemia was present was probably due to the fact that the hyperglycemia in these cases was too small to produce such an elimination of dextrose into the alimentary canal. Blumenthal[11] has shown that relatively large quantities of dextrose can be injected intravenously before a glycosuria appears. When we consider how much more readily sugar is eliminated through the kidneys than into the intestine, we may well assume that still larger quantities of dextrose have to be injected intravenously before an elimination into the intestine takes place. The fact that a certain degree of hyperglycemia is sufficient to cause a glycosuria is not yet evidence that the same degree is also sufficient to cause an excretion into the intestine. The very fact that the quantity of dextrose in the urine in Fischer and Moore's experiment amounted only to about 11 per cent. of the injected dextrose, while the dextrose in the urine in our successful experiments was three or four times that percentage, shows that in their experiment the hyperglycemia must have been of a low degree.

While we thus agree in general with MacCallum that the elimination of dextrose into the intestine is dependent upon a hyperglycemia, we can not agree with his view that this elimination constitutes an "intestinal diabetes," and still less can we look upon the slight increase in the excretion of sugar into the alimentary canal as a "vicarious action"; that is, that in the absence of the kidneys, the intestine is capable of assuming the excretory activities of those eliminating organs. Without entering here upon the large question of vicarious activity of organs, we must insist that this assumption is especially inapplicable in the case under discussion. The average percentage of elimination into the intestine of the injected dextrose amounts, for the six normal rabbits which received seven grams per kilo. of body weight, to 1.2 per cent. (table IV). For the nephrectomized rabbits receiving the same quantities of dextrose, the average percentage amounts to 2.2 per cent (table V); that is, the elimination into the intestine of nephrectomized rabbits was increased by 1 per cent. The elimination of the injected dextrose through the kidneys in the rabbits with intact kidneys amounts to 40.9 per cent.

[11] Blumenthal, *Beitr. z. chem. Physiol. u. Pathol.*, 1905, vi, 329.

A theory which assumes that an increase of elimination into the intestine of nephrectomized rabbits of 1 per cent. means a functional compensation for the loss of elimination of 40.9 per cent. is so unsatisfactory that it seems to require no special discussion. We must remember, at the same time, that this increase of 1 per cent. is contributed by the rather large area of the mucous membrane of the entire small intestine. The plausible interpretation of the increase after nephrectomy is that in animals which have received considerable quantities of dextrose intravenously, in consequence of the absence of the specifically eliminating organs, some increase in the elimination of the constituents of lymph and blood occurs in all glands and over the surfaces of all the mucous membranes. The eliminated quantities, however, are usually too small to be readily recognized, except when they are collected from such a large surface as the mucous membrane of the entire small intestine.

SUMMARY.

The contents of the stomach and small intestine of normal rabbits, kept on a diet poor in easily convertible carbohydrates, when removed immediately after killing the animal (by clamping the trachea), usually contain a very small but measurable amount of dextrose (reducing substances). A preceding nephrectomy does not increase the amounts of the dextrose in these viscera.

An intravenous injection of dextrose, if given in a sufficient quantity in a unit of time, causes a definite excretion, or increase of excretion, into the small intestine and the stomach. The amount of excretion, however, is incomparably smaller than the amount eliminated through the kidneys.

A preceding double nephrectomy increases the gastro-intestinal elimination of the intravenously injected dextrose. The increase, however, is far too small to be considered in the light of a functional compensation for the loss of the considerable elimination through the kidneys.

In conclusion, I desire to express my thanks to Dr. S. J. Meltzer for constant and invaluable aid and advice during this investigation.

ON THE MECHANISM OF CHLORIN RETENTION IN PNEUMONIA.*

By FLORENTIN MEDIGRECEANU.

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It is a well established fact that chlorin is retained in pneumonia.[1] Since in this disease an exudate is formed, one might suspect that the retention of chlorin would be associated with it. However, the most striking and interesting point has been that in most of the cases which have been carefully studied, the quantity of the chlorin retained has exceeded that calculated for and found in the exudate. It is therefore evident that part of the chlorin is stored elsewhere in the body than in the exudate. The location of the place where the remainder of the retained chlorin is stored has been the object of many subsequent investigations. The final conclusion now generally accepted is that the retention takes place throughout the fixed tissues of the body.

The next question to be studied is the mechanism of the chlorin retention. The point especially treated in this paper is, whether the conditions of permeability of the kidney may be responsible for the retention, or whether the storing up is due to an active process in the tissues.

This question has been treated in a very satisfactory manner, especially by Hutchison,[2] and more recently by von Hösslin and Kashiwado.[3] Although these investigators concluded that the kidney plays an insignificant rôle, if any, in the process of chlorin retention in pneumonia, nevertheless it seemed of interest to control their conclusions experimentally by making a comparative study of

* Received for publication, June 28, 1911.

[1] A complete discussion of the question may be found in the paper of Hutchison, *Jour. Path. and Bact.*, 1898, v, 407; and of von Hösslin, *Deutsch. Arch. f. klin. Med.*, 1908, xciii, 404.

[2] Hutchison, *Jour. Path. and Bact.*, 1898, v, 422.

[3] von Hösslin and Kashiwado, *Deutsch. Arch. f. klin. Med.*, 1911, cii, 520.

the chlorin metabolism in three series of animals: namely (1) animals with experimental pneumonia; (2) animals with general pneumococcal infection; and (3) animals in which a pleural exudate had been produced by chemical means, *i. e.*, turpentine. Series 1 and 3 were carried out on dogs; series 2, on rabbits.[4]

Pneumonia in dogs was produced by the method of Lamar and Meltzer.[5] This consists in introducing five to ten cubic centimeters of a broth culture of virulent pneumococci through the larynx into a bronchus by means of a small stomach tube.

Turpentine pleurisy in dogs was induced by employing the technique of Opie[6] and that of Manwaring[7] for obtaining leucocytes. One to two cubic centimeters of turpentine are injected into the pleural cavity at intervals of three days. By removing the first exudate the day following the second injection, a new one accumulates very rapidly. This second exudate contains, as a rule, a much larger number of leucocytes than the first one. Thus the opportunity is given of studying two different aspects of pleurisy in the same animal.

It may also be mentioned that in all the experiments with the pneumococcus here described, the same strain of organisms was used, *i. e.*, the Neufeld strain, which is the one generally employed in the Rockefeller Institute.

SUMMARY AND CONCLUSION.

The following conclusions may be drawn from an analysis of the protocols:

1. The chlorin output is very much diminished during the process of experimental pneumonia in dogs (tables I and II). The quantity of chlorin retained in the cases with well developed exudates (table I) is much larger than the exudate could account for.

2. Turpentine pleurisy in dogs (tables III and IV) also is associated with a retention of chlorin, but this is not so marked as in

[4] A short remark on chlorin metabolism in rabbits with general pneumococcal septicemia is also made by Hutchison, *Jour. Path. and Bact.*, 1898, v, 431.

[5] Lamar and Meltzer, *Proc. Soc. Exper. Biol. and Med.*, 1910, vii, 102.

[6] Opie, *Jour. Exper. Med.*, 1908, x, 423.

[7] Personal communication.

TABLE I[1].

Dog A. Experimental Pneumonia.

Date, 1911.	Body weight in kilos.	Urine. In c.c.	Urine. Total nitrogen in grams.	Urine. Chlorin in grams.	Feces. In grams.	Feces. Total nitrogen in grams.	Feces. Chlorin in grams.	Rectal temperature, C.	Leucocytes in 1 cu. mm.	Remarks.
April 15.	10.760	300	5.010	2.375						
April 16.	10.660	330	5.700	2.375						
April 17.	10.600	350	5.740	2.440	21.88	1.290	0.024	38.0	9,000	No albumin in the urine.
April 18.	10.320	620	6.740	2.510				40.0	2,500	8 c.c. pneumococcus culture into the trachea; dyspnea and cough; dullness over the right lower lobe of the lung; bronchial breathing.
April 19.	10.100	320	4.960	0.419				39.5	2,000	No intake of food.
April 20.	9.860	220	5.640	0.038				38.7	10,600	
April 21.	9.640	220	4.980	0.080	11.55	0.581	0.016	38.5	10,000	The food for this day contained only 4 gm. nitrogen and 2 gm. chlorin.
April 22.	9.760	230	5.550	0.053				38.5	14,000	
April 23.	9.820	240	5.730	0.966				38.4	14,000	The urine contained traces of albumin.
April 24.	9.860	250	4.760	1.870				38.2	19,000	
April 25.	9.840	260	5.110	2.30	11.6	0.600	0.025	38.2		Physical signs of pneumonia have almost disappeared.
April 26.	9.840	320	5.256	2.27				38.0		

Daily diet { 100 gm. beef heart; 100 gm. cracker meal; 1.254 gm. chlorin as sodium chlorid solution; distilled water } { total nitrogen 4.900 gm.; total chlorin 2.374 gm. }

[1] The daily quantities of urine were obtained from dogs by means of catheterization, from rabbits by gently pressing over the bladder.

The nitrogen was estimated by the Kjeldahl-Gunning method.

The chlorin was determined in the urine by Volhard's method; in the feces and the food after incineration in the usual way.

TABLE II.

Dog B. Experimental Pneumonia.

Date, 1911.	Body weight in kilos.	Urine.			Feces.			Rectal temperature, C.	Remarks.
		In c.c.	Total nitrogen in grams.	Chlorin in grams.	In grams.	Total nitrogen in grams.	Chlorin in grams.		
Mar. 12.	12.640	320	7.03	1.650	12.230	0.870	0.015	38.0	No albumin in the urine.
Mar. 13.	12.600	320	6.72	1.730				38.0	
Mar. 14.	12.300	800	8.29	2.620				39.5	10 c.c. pneumococcus culture into the trachea. No dullness or bronchial breathing could be detected.
Mar. 15.	12.440	340	7.30	0.671	15.060	1.055	0.018	38.5	
Mar. 16.	12.580	400	8.96	2.384				38.0	
Mar. 17.	12.500	390	7.25	2.040	11.130	0.803	0.012	38.0	
Mar. 18.	12.480	340	6.89	1.720				38.0	

Daily diet { 200 gm. beef heart; 80 gm. cracker meal; 1.080 gm. chlorin as sodium chlorid solution; distilled water } { total nitrogen 7.373 gm.; total chlorin 2.077 gm. }

TABLE III.

Dog C. Experimental Pleurisy.

Date, 1911.	Body weight in kilos.	Urine.			Feces.			Rectal temperature, C.	Remarks.
		In c.c.	Total nitrogen in grams.	Chlorin in grams.	In grams.	Total nitrogen in grams.	Chlorin in grams.		
Feb. 19.	12.540	310	6.460	1.715	8.620	0.563	0.011	38.0	No albumin in the urine.
Feb. 20.	12.540	980	9.630	1.842				38.7	1 c.c. turpentine into the right pleural cavity.
Feb. 21.	12.600	880	9.370	1.357				38.5	
Feb. 22.	12.600	600	6.580	2.206	10.020	0.726	0.018	38.0	
Feb. 23.	13.000	380	8.570	1.698				39.5	1 c.c. turpentine into the right pleural cavity. Dog vomited.[1]
Feb. 24.	12.280	1500	11.960	0.532	5.140	0.352	0.011	39.0	500 c.c. exudate taken out.[2]
Feb. 25.	12.200	950	9.520	0.907				39.0	80 c.c. exudate taken out.[3]
Feb. 26.	12.380	440	8.080	1.390				38.7	The urine contained traces of albumin.
Feb. 27.	12.400	450	7.830	2.050	9.570	0.723	0.013	38.5	
Feb. 28.	12.300	450	7.780	2.720				38.3	
Mar. 1.	12.100	400	7.610	2.930				38.0	

Daily diet { 200 gm. beef heart; 80 gm. cracker meal; 1.080 gm. chlorin as sodium chlorid solution; distilled water } total nitrogen = 7.373 gm. total chlorin = 2.077 gm.

[1] The vomitus contained 1.578 gm. nitrogen and 0.77 gm. chlorin.

[2] This exudate contained 0.808 per cent. nitrogen and 0.360 per cent. chlorin.

[3] This exude contained 0.680 per cent. nitrogen and 0.366 per cent. chlorin.

TABLE IV.

Dog D. Experimental Pleurisy.

Date, 1911.	Body weight in kilos.	Urine.			Feces.			Rectal temperature, C.	Leucocytes in 1 cu. mm.	Remarks.
		In c.c.	Total nitrogen in grams.	Chlorin in grams.	In grams.	Total nitrogen in grams.	Chlorin in grams.			
Mar. 16.	19.340	650	9.500	3.320						
Mar. 17.	19.500	500	9.502	3.270	22.170	1.475	0.025	38.0	7,300	Urine free from albumin.
Mar. 18.	19.700	570	12.270	2.115				38.0	75,000	1.5 c.c. turpentine into the right pleural cavity.
Mar. 19.	19.600	940	13.230	3.325				39.0	18,600	
Mar. 20.	19.700	640	7.260	3.165	15.950	1.020	0.025	39.0	23,300	
Mar. 21.	20.000	580	11.330	2.140				39.2	29,500	1 c.c. turpentine into the right pleural cavity.
Mar. 22.	20.000	390	12.230	0.391	13.500	0.858	0.012	39.2	29,000	250 c.c. exudate taken out.[1]
Mar. 23.	19.000	980	12.270	0.503	12.700	0.810	0.012	39.0	25,600	420 c.c. exudate taken out.[2]
Mar. 24.	18.400	580	9.700	1.460				39.0	23,500	
Mar. 25.	18.000	420	7.530	0.716	7.980	0.561	0.006	39.2	25,000	The dog took no food except distilled water.
Mar. 26.	17.400	360	7.720	0.861					24,000	
Mar. 27.	17.100	240	6.160	0.443				39.0		The urine contained traces of albumin.
Mar. 28.	17.000	240	5.290	0.770				39.0	22,000	
Mar. 29.	16.600	270	5.200	0.528				39.0		Killed.

Daily diet { 200 gm. beef heart; 150 gm. cracker meal; 30 gm. lard; 1.849 gm. chlorin as sodium chlorid solution; distilled water } { total nitrogen = 8.620 gm.; total chlorin = 3.460 gm. }

[1] The exudate contained in 1 cu. mm. 13,500 leucocytes and 20,000 erythrocytes.
Nitrogen = 0.768 per cent.
Chlorin = 0.389 per cent.

[2] The exudate contained in 1 cu. mm. 63,000 leucocytes and 44,000 erythrocytes.
Nitrogen = 0.666 per cent.
Chlorin = 0.365 per cent.

TABLE V.

Rabbit M. Pneumococcal Septicemia.

Date, 1911.	Body weight in grams.	Urine.			Feces.			Rectal temperature, C.	Leucocytes in 1 cu. mm.	Remarks.
		Approximate content in c.c.	Total nitrogen in grams.	Chlorin in grams.	In grams.	Total nitrogen in grams.	Chlorin in grams.			
May 5.	2,470	70	0.764	0.697						Urine free from albumin.
May 6.	2,500	110	0.705	1.040						
May 7.	2,500	100	0.754	0.964						
May 8.	2,470	100	0.796	1.118						
					7.25	0.225	0.076	39.0	9,500	
May 9.	2,260 (dead)	25	0.211	0.173				40.0	8,300	3 hours: After intravenous inoculation of 1 c.c. of a broth culture of virulent pneumococcus.
		150	0.602	1.297				41.3	1,000	20 hours: The urine contained much albumin.
		40	0.058	0.153						24 hours: Numerous pneumococci were present in the blood.
	Total	215	0.871	1.623						

Daily diet: 50 gm. bread; 250 gm. cabbage; 0.627 gm. chlorin as sodium chlorid solution into the stomach — total nitrogen = 1.400 gm.; total chlorin = 1.092 gm.

TABLE VI.

Rabbit F. Pneumococcal Septicemia.

Date, 1911.	Body weight in grams.	Urine.			Feces.			Rectal temperature, C.	Leucocytes in 1 cu. mm.	Remarks.
		Approximate content in c.c.	Total nitrogen in grams.	Chlorin in grams.	In grams.	Total nitrogen in grams.	Chlorin in grams.			
May 5.	2,720	70	1.275	0.136						
May 6.	2,900	65	0.935	0.421	17.15	0.659	0.039			
May 7.	3,040	65	0.936	0.577						
May 8.	3,140	115	1.085	0.840						
May 9.	3,070	150	0.917	1.140	8.58	0.337	0.029	39.0	12,800	
May 10.	2,570	50	0.332	0.451				40.0	5,200	7 hours
		150	0.591	1.109				41.4	2,800	15 hours
		140	0.336	0.586						20 hours
		340	1.259	2.146						After intravenous inoculation of 1 c.c. of blood containing pneumococci (from Rabbit M, table V).
May 11.	2,460 (dead)	100	0.570	0.592						33 hours The urine contained much albumin. Numerous pneumococci were present in the blood.

Daily diet { 250 gm. cabbage; 50 gm. bread; 0.627 gm. chlorin as sodium chlorid solution into the stomach } total nitrogen = 1.400 gm. total chlorin = 1.092 gm.

pneumonia. There is no direct quantitative relation between the water output and the chlorin excretion.

3. The rabbits with pneumococcal septicemia (tables V and VI) did not retain chlorin in any phase of the disease, a fact which Hutchison has also noticed.

It seems justifiable to conclude that the retention of chlorin in the organism in the course of pneumonia is conditioned by more than one factor. The formation of the exudate is one of these factors, but it alone is not capable of producing the same degree of chlorin retention as occurs in pneumonia. The additional factors are not yet determined; but it may be stated that the retention of chlorin is probably not due to the general effect of the pneumococcus on the organism or to its injury to the kidney. The principal cause of the retention seems, therefore, to be due to local changes associated with the pulmonary condition.

ON THE COMPOSITION OF URINARY ALBUMIN.*

By FLORENTIN MEDIGRECEANU.

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Since the studies of Brown-Sequard, Teissier, L. Brunton, and Hoffman,[1] a great deal of work has been done on urinary albumin, in order to differentiate various kinds of proteins, to demonstrate their origin, and their diagnostic and prognostic significance.

The attempts at identification have been chiefly by means of the physical or biological properties; relatively few chemical analytical data are found in the literature. One method of identification, which seems undoubtedly superior to the earlier ones employed, consists in analyzing the urinary protein for the amino-acids contained in it. This method is not a new one. Magnus-Levy[2] studied the partition of nitrogen in Bence Jones' protein in the urine of one case, and Abderhalden and Rostoski[3] demonstrated the presence of most of the known amino-acids in Bence Jones' protein from another case. In a later paper, Abderhalden[4] points out the importance of studying the quantitative and qualitative distribution of amino-acids in urinary albumin.

Recently Van Slyke[5] has worked out an analytical method which allows one to obtain a fairly complete picture of the proportions in which the different types of amino-acids enter into the composition of a protein. A great advantage of the method is the possibility of using only two to three grams of material for each analysis. This

* Received for publication, June 25, 1911.

[1] See von Noorden, Handbuch der Pathologie des Stoffwechsels, Berlin, 1906, i, 1008; Ellinger, Handbuch der Biochemie, hsg. von C. Oppenheimer, Berlin, 1910, iii, 654.

[2] Magnus-Levy, *Ztschr. f. physiol. Chem.*, 1900, xxx, 200.

[3] Abderhalden and Rostoski, *Ztschr. f. physiol. Chem.*, 1905, xlvi, 125.

[4] Abderhalden, *Ztschr. f. exp. Path. u. Therap.*, 1905, ii, 642.

[5] Van Slyke, *Ber. d. deutsch. chem. Gesellsch.*, 1910, xliii, 3710; *Jour. Biol. Chem.*, 1911, ix, 185.

method is based on the fact that amino-groups react with nitrous acid, giving off nitrogen as gas, which can be measured volumetrically.

By determining the total nitrogen and the amino-nitrogen in a solution of amino-acids, and by making the same estimation after precipitation with phosphotungstic acid in both the precipitate and the filtrate, and by using special methods for arginin and cystin, it is possible to obtain, under definite conditions that permit of quantitative comparisons, many important data for differentiating various kinds of proteins. A few main details, illustrating the conclusions that can be drawn from the analytical results obtained by this method, may be mentioned.

It has been shown that all amino-acids react with all their nitrogen under the influence of nitrous acid, except trytophan, which reacts with one half, histidin with one third, arginin with one fourth, and prolin and oxyprolin with none. The following table shows these relations in percentages; at the same time the precipitable and non-precipitable amino-acids should be kept in mind.

Precipitable by phosphotungstic acid	Lysin reacts with 100 per cent. of its total nitrogen.
	Cystin reacts with 107 per cent. of its total nitrogen.
	Histidin reacts with 33.3 per cent. of its total nitrogen.
	Arginin reacts with 25 per cent. of its total nitrogen.
Non-precipitable by phosphotungstic acid	Prolin reacts with 0 per cent. of its total nitrogen.
	Oxyprolin reacts with 0 per cent. of its total nitrogen.
	Tryptophan reacts with 50 per cent. of its total nitrogen.
	The others react with 100 per cent. of their total nitrogen.

Considering the relation of amino-nitrogen to total nitrogen in a mixture of phosphotungstic precipitable and non-precipitable amino-acids, as they occur in proteins that undergo complete hydrolysis, a high percentage would indicate a relatively low amount of amino-acids reacting with part or none of their nitrogen (histidin, arginin, prolin, oxyprolin, tryptophan). The contrary holds good in the case of a low percentage. Further details may be obtained by comparing the ratio of amino-nitrogen to total nitrogen in both the phosphotungstic precipitate and filtrate. High figures in the precipitate point to a predominance of lysin and cystin; high figures in the filtrate indicate a predominance of amino-acids reacting with all of their nitrogen and *vice versa*.

With these details in mind, I have applied Van Slyke's method in the analysis of a series of urinary albumins. Some of these urines were obtained from dogs that had been poisoned with uranium nitrate or uranium nitrate and phosphorous oil, and others from cases of acute and chronic nephritis in man. The latter urines were obtained through the kindness of Dr. Wachsmann from the Montefiore Home, New York, and of Dr. Wilson from the Willard Parker Hospital, New York. The object of this work was to obtain a general orientation in the problem and not to make an exhaustive study of a single limited question.

Phosphorous oil was injected together with uranium nitrate, since under the influence of phosphorus the degenerating liver loses chiefly its protoplasmic constituents, in which the hexon bases, histidin, arginin, and lysin, are relatively more abundant than in the nuclei, as Wakeman[6] has shown. Should such a protein with a large amount of hexon bases be liberated from the protoplasm of this organ and carried into the blood stream, one would expect *a priori* that it might pass the permeable kidney and be found in the urine.

The protocols here given contain a series of representative analyses. With regard to the technical details, in every case the protein was quantitatively precipitated in the filtered urine by heat and acetic acid in the usual way; the precipitate was centrifugalized and washed thoroughly with water and alcohol, and then dried. Usually 3 to 4 gm. of the dried substance were completely hydrolyzed by boiling for twenty hours with 250 c.c. of 20 per cent. hydrochloric acid. The solution was filtered, made up to 500 c.c., the total nitrogen determined by the Kjeldahl-Gunning method in 2 portions of 10 c.c., and the remainder evaporated on the water bath to syrupy consistency. The ammonia was then liberated with lime and redistilled at 40° C. *in vacuo* into N/10 sulphuric acid. The precipitate of lime, containing most of the melanin, was removed by filtration and washed thoroughly. The remainder of calcium hydroxid in the filtrate was precipitated with carbonic acid, and the clear solution was concentrated to 100 c.c. *in vacuo*. Two portions of 5 c.c. of this solution were used for total nitrogen determination, and two other 5 c.c. portions for amino-nitrogen estimation in Van Slyke's apparatus. From the remaining solution, a definite quantity containing a known amount of nitrogen was then precipitated at 90° C. with phosphotungstic and hydrochloric acids, the following figures being used as standards: for 0.3 gm. nitrogen, 15 gm. phosphotungstic acid, 7 gm. hydrochloric acid, and a dilution of 200 c.c. For quantities of nitrogen larger or smaller than 0.3 gm., the figures were modified according to the amount taken. The mixture was kept at room temperature for at least sixty hours and the phosphotungstic precipitate was then filtered and washed twelve times with 10 c.c. of a solution containing 2.5 per cent.

[6] Wakeman, *Ztschr. f. physiol. Chem.*, 1905, xliv, 335.

phosphotungstic acid and 3.5 per cent. hydrochloric acid, as described by Van Slyke.[1] The precipitate was decomposed with sodium hydroxid solution, phenolphthalein being used as indicator. Care was taken that the alkali exceeded the acid only by a few drops. This slightly alkaline solution was concentrated *in vacuo* at 40° C. to a volume of 50 c.c., and its total nitrogen and amino-nitrogen were determined. The phosphotungstic filtrate was neutralized with sodium hydroxid, concentrated to 200 c.c., and the total nitrogen and the amino-nitrogen in it were likewise estimated.

On analyzing the figures obtained, the question arises as to whether the albumin isolated in the manner described above was pure. A series of controls was made in order to obtain an idea of the extent of error caused by impurities mixed with the urinary albumin. Various samples were divided into two portions; one sample was merely centrifugalized, while the other was thoroughly washed with water and alcohol. The differences resulting concerned themselves chiefly with the quantity of ammonia, which was derived, undoubtedly, chiefly from urea. Other variations were so small that they fell within the limits of error. Such an example is shown in table III. On account of this possibility, all the ammonia estimations were discarded and the percentages made from the determinations of nitrogen obtained after distilling the ammonia and filtering off the precipitate of lime and melanin.

SUMMARY AND CONCLUSIONS.

A few findings which seem to be of importance may be pointed out:—

Table I shows the analytical figures of serum-albumin, serum-globulin, and fibrin of the normal dog. The main difference between albumin and globulin appears in the relation of the precipitable to the non-precipitable total nitrogen and amino-nitrogen. Precipitable total nitrogen as well as amino-nitrogen is considerably larger in the albumin than in the globulin.

In the cases of uranium nitrate nephritis (table II), the important figures approximate very closely those of normal serum-albumin.

The samples from dog 3, that had been poisoned at the same time with phosphorous oil and uranium nitrate, show relatively large variations as compared with the figures from specimens from the

[1] Van Slyke, *Ber. d. deutsch. chem. Gesellsch.*, 1910, xliii, 3177.

TABLE I[1].

Serum-albumin, Serum-globulin, and Fibrin[2] from the Normal Dog.

Substance.	Amount of nitrogen hydrolyzed, in grams.	Ammonia nitrogen.		Nitrogen analyzed after removing ammonia and melanin. In grams.	Total amino-nitrogen.		Nitrogen used for precipitation. In grams.	Phosphotungstic precipitate.					Phosphotungstic filtrate.				
								Total nitrogen.		Amino-nitrogen.			Total nitrogen.		Amino-nitrogen.		
		In grams.	Per cent. of total nitrogen.		In grams.	Per cent. of the total nitrogen.		In grams.	Per cent.	In grams.	Per cent. of the total amino-nitrogen.	Per cent. of the total precipitable nitrogen.	In grams.	Per cent.	In grams.	Per cent. of the total amino-nitrogen.	Per cent. of the total filterable nitrogen.
Albumin .	0.3985	0.0283	7.15	0.3330	0.2745	82.5	0.2540	0.0798	31.2	0.0466	21.0	58.4	0.1848	69.8	0.1742	79.0	94.3
Globulin .	0.4508	0.0411	9.15	0.4010	0.3320	82.8	0.2955	0.0715	24.1	0.0402	16.2	56.3	0.2240	75.9	0.2080	83.8	92.8
Fibrin . . .	0.4975	0.0474	9.57	0.4198	0.3535	84.4	0.3014	0.0844	28.0	0.0484	19.7	57.3	0.2170	72.0	0.2042	80.3	94.2

[1] All of the analyses in the three tables here given were done in duplicate.

[2] The albumin and globulin from the serum of five normal dogs were separated by means of magnesium sulphate in the usual way; then both proteins were coagulated by heat and acetic acid, centrifugalized, and washed thoroughly with water and alcohol. The fibrin was isolated by whipping the blood and was washed in a current of water.

TABLE II.

Urinary Albumin from Dogs with Experimental Nephritis.

Dogs 1 (17 kilos.) and 2 (18 kilos.) were poisoned with 0.02 gm. uranium nitrate given once subcutaneously. Dogs 3 (2 kilos.) and 4 (19 kilos.) were each given subcutaneously at the same time 0.006 gm. uranium nitrate and 3 c.c. phosphorous oil; three days later they were given 0.002 gm. uranium nitrate and 5 c.c. phosphorous oil. Specimens (*a*) were collected after the first inoculation; specimens (*b*) were collected after the second inoculation. Dog 3 survived; dog 4 died 30 hours after the second injection.

Number of dog.	Nitrogen analyzed after removing ammonia and melanin. In grams.	Total amino-nitrogen.		Nitrogen used for precipitation. In grams.	Phosphotungstic precipitate.					Phosphotungstic filtrate.				
					Total nitrogen.		Amino-nitrogen.			Total nitrogen.		Amino-nitrogen.		
		In grams.	Per cent. of the total nitrogen.		In grams.	Per cent.	In grams.	Per cent. of the total amino-nitrogen.	Per cent. of the total precipitable nitrogen.	In grams.	Per cent.	In grams.	Per cent. of the total amino-nitrogen.	Per cent. of the total filterable nitrogen.
1	0.3680	0.2940	79.9	0.2803	0.0840	30.0	0.0496	21.6	59.0	0.1963	70.0	0.1792	78.4	91.70
2	0.3080	0.1813	80.9	0.1749	0.0511	29.3	0.0299	21.4	58.5	0.1238	70.7	0.1095	78.6	88.50
3*a*	0.4205	0.3580	84.7	0.3150	0.0945	30.0	0.0630	24.0	63.9	0.2205	70.0	0.2000	76.0	90.75
3*b*	0.5400	0.4521	83.8	0.3944	0.1177	29.9	0.0746	22.3	63.5	0.2767	70.1	0.2600	77.7	93.90
4*a*	0.3390	0.2687	79.2	0.2599	0.0767	29.5	0.0451	21.5	58.8	0.1832	70.5	0.1650	78.5	90.10
4*b*	0.5205	0.4185	80.4	0.3946	0.1176	29.7	0.0701	21.8	59.8	0.2770	70.3	0.2515	78.2	90.80

TABLE III.

Urinary Albumin from Cases of Nephritis in Man.

No. 1. Acute nephritis (scarlet fever). Specimen (*a*) was collected in January, 1911. Specimen (*b*) was collected in February, 1911.

Nos. 2 to 5. Chronic nephritis. Case 2 was a patient with Pott's disease. Specimens 3 and 6 are from patients with the usual form of chronic nephritis. Specimen 4 is from a patient with diabetes mellitus. Specimen 5 is from a patient with gout.

No. 6. (*a*) represents the analysis of a thoroughly washed albumin. (*b*) represents the analysis of a merely centrifugalized albumin.

Number of case.	Nitrogen analyzed after removing ammonia and melanin. In grams.	Total amino-nitrogen.		Nitrogen used for precipitation. In grams.	Phosphotungstic precipitate.					Phosphotungstic filtrate.				
					Total nitrogen.		Amino-nitrogen.			Total nitrogen.		Amino-nitrogen.		
		In grams.	Per cent. of the total nitrogen.		In grams.	Per cent.	In grams.	Per cent. of the total amino-nitrogen.	Per cent. of the total precipitable nitrogen.	In grams.	Per cent.	In grams.	Per cent. of the total amino-nitrogen.	Per cent. of the total filterable nitrogen
1*a*	0.3920	0.3080	78.5	0.3010	0.0930	30.9	0.0518	22.3	55.7	0.2080	69.1	0.1792	77.7	86.8
1*b*	0.4228	0.3420	80.9	0.3254	0.0974	29.9	0.0566	21.0	58.1	0.2280	70.1	0.2101	79.0	92.3
2	0.4190	0.3170	75.6	0.3127	0.0932	29.8	0.0512	21.4	55.0	0.2195	70.2	0.1887	78.6	86.0
3	0.4506	0.3541	78.7	0.3380	0.1085	32.2	0.0638	24.1	58.7	0.2295	67.8	0.2010	75.9	87.5
4	0.4592	0.3785	82.4	0.3530	0.1156	32.8	0.0676	23.4	58.5	0.2374	67.2	0.2210	76.6	93.1
5	0.3600	0.2880	80.0	0.2708	0.0837	30.8	0.0474	22.3	56.6	0.1871	69.2	0.1871	77.7	88.3
6*a*	0.4708	0.3785	80.4	0.3595	0.1120	31.1	0.0674	23.5	60.2	0.2475	68.9	0.2185	76.5	88.3
6*b*	0.4030	0.3165	78.5	0.3085	0.0946	30.7	0.0568	23.2	60.1	0.2139	69.3	0.1878	76.8	87.9

other dogs, chiefly as regards the amino-nitrogen distribution: *i. e.*, in dog 3, (1) the amount of amino-nitrogen to the total nitrogen in the solution before precipitation is higher; (2) the percentage of precipitable amino-nitrogen is larger; and (3) the ratio of precipitable amino-nitrogen to precipitable total nitrogen exceeds that of the other cases.

All these changes, together with the fact that the total precipitable nitrogen did not undergo any quantitative variation, suggest that in the case of dog 3 the analyzed material contained a higher amount of lysin or cystin.

It may further be mentioned that the analytical figures in this case differ also from those of the normal serum-albumin and still more from those of the serum-globulin. These changes, however, were not found in the case of dog 4, although this animal was treated in the same manner as the preceding dog.

In the cases of nephritis in man (table III), striking differences were met with in the case of acute scarlet fever nephritis (No. 1*a*) and in the case (No. 2) of a patient with chronic nephritis and Pott's disease. This patient died a few weeks after the specimen for analysis was collected. The autopsy showed a general amyloidosis.

The variations in both cases consist in a lowering of the ratio of amino-nitrogen to total nitrogen in the solution before precipitation, and corresponding to this, a fall of the same ratio in the filterable nitrogen. Such a change points to a relatively larger amount of prolin and oxyprolin or tryptophan in these cases.

As a whole, one may conclude that Van Slyke's method, carefully applied and sufficiently controlled, may also be used for the study of urinary albumin. The results already obtained indicate that definite differences in the composition of urinary "albumin" may be detected. As yet it is premature to establish a definite relationship between the chemical composition of the "albumin" and the clinical or pathological conditions under which it appears, but it seems hopeful that further work may lead to the finding of such a relationship.

A PHYSIOLOGICAL STUDY OF EXPERIMENTAL NEPHRITIS DUE TO BACTERIAL POISONS AND CYTOTOXIC SERA.*

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In an earlier communication[1] we have described the vascular reactions characteristic of those forms of nephritis in the dog caused by the administration of various powerful renal irritants. Therein were discussed forms of nephritis in which either tubular or vascular changes predominated; the nephritides due to uranium nitrate, potassium chromate, and corrosive sublimate represented the first and those caused by arsenic and cantharidin, the second type. It was found, however, that although either a tubular or vascular lesion might be the predominating characteristic of a nephritis due to a given poison, it was impossible to conclude that a nephritis was purely vascular or purely tubular; and moreover it was shown that a tubular nephritis might pass into a stage closely resembling, if not identical with, the vascular type. This inability to demonstrate experimentally purely vascular or epithelial types of the affection is in accord with our knowledge that although in one form of nephritis in man the most striking lesion may be in the glomeruli, in another it may be in the tubular epithelium, and in neither is the evidence of kidney injury limited to the most apparent lesion. Experimental nephritis, however, cannot be brought absolutely into relation with the nephritides of man, because in the latter the powerful chemical agents used to produce the experimental lesions are seldom concerned etiologically, and the lesions which these cause, in so far at

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[1] Pearce, R. M., Hill, M. C., and Eisenbrey, A. B., Experimental Acute Nephritis, The Vascular Reactions and the Elimination of Nitrogen, *Jour. Exper. Med.*, 1910, xii, 196.

least as the metallic salts are concerned, are of a type seldom seen in man. We have therefore, in the present investigation, attempted the study of forms of experimental nephritis more nearly analogous in etiology and anatomical changes to those occurring in man. For this purpose diphtheria toxin has been employed as representing the etiological factor in a typical variety of nephritis of bacterial origin. Mallein and tuberculin have also been used as additional bacterial poisons. Certain cytotoxic sera, on account of the peculiar action on the kidney caused by their nephrotoxic and hemolytic properties, have likewise been utilized.

METHODS.

The methods employed are similar to those used in the earlier study and are, with slight modifications, based on the procedures described by Schlayer and Hedinger.[2] Briefly stated these include a simultaneous kymographic record of the blood pressure, the volume of the kidney and the flow of urine. The blood pressure was taken from the left femoral artery with a mercury manometer; changes in the kidney volume were determined by the use of a gutta-percha capsule connected with a bellows recorder; and the flow of urine, obtained from a cannula in the bladder, was registered by an electrical drop-recorder. For testing the power of contraction of the renal vessels, adrenalin (Parke, Davis and Co.) in doses of one drop of the 1 to 1000 solution was used; and for testing the power of dilatation and diuresis, caffein or sodium chloride solution or both were employed. The caffein was used in the proportion of two cubic centimeters of a one per cent. solution, and the sodium chloride in the proportion of five cubic centimeters of a five per cent. solution per kilo. of body weight. Dog urine was used in doses of three cubic centimeters for the purpose of rapidly lowering the blood pressure and of testing the efficiency of the oncometer. All the fluids were introduced through a cannula in the right femoral vein, due regard being taken of their temperature and speed of injection.

All experiments were performed under complete ether anesthesia.

[2] Schlayer and Hedinger, Experimentelle Studien über toxische Nephritis, *Deutsch. Arch. f. klin. Med.*, 1907, xc, 1.

Spontaneous nephritis and other accidental lesions of the kidney were eliminated by a preliminary period of observation. Alterations in the physiology of the kidney were eventually correlated with the anatomical lesions and the changes in the urine.

THE EFFECT OF DIPHTHERIA TOXIN.

Schlayer and Hedinger found that, in the rabbit, diphtheria toxin produced, as far as the vascular reactions were concerned, a type of nephritis lying midway between the typically tubular and the typically vascular. In the early stages the lesion was apparently tubular and in its later stages certainly vascular in character. The toxin which they used (Höchster Farbwerken 1½ fach normal Toxin) was injected subcutaneously as well as intravenously in doses of 0.25 to 1 cubic centimeter, and it caused albumin to appear within a few hours and casts within twenty-four hours; death occurred suddenly much as in cantharidin poisoning. Up to nine hours after injection the vascular reactions remained unaltered; the power of the kidney vessels to dilate and contract was unchanged and diuresis scarcely affected, although at the end of nine hours the urine contained a large amount of albumin, an occasional hyaline cast and a few erythrocytes. At twelve hours, though the vessels still reacted to stimuli, diuresis was lessened and the blood pressure indicated the beginning of general poisoning. At twenty-four hours diuresis and all vascular responses either failed or were minimal, and the kidney showed no pulsation. The last condition is obviously that of an overwhelming intoxication, and Schlayer and Hedinger produced it purposely in order to obtain the maximum effect on the kidney.

The object of our experiments was to produce at the outset, if possible, less severe lesions, the gradually increasing severity of which might be followed accurately. The toxin used was kindly supplied by Dr. W. H. Park, of the Research Laboratories of the New York City Board of Health. Its toxicity was approximately 1/800; that is, 1/800 cubic centimeter was sufficient to kill a 250 gram guinea-pig in four to five days; the exact lethal dose, however, was not determined.

The study of the effect of this toxin was made on ten dogs. Early in the work it was found that doses of 0.5 to 1 cubic centimeter caused death in dogs weighing from nine to eleven kilos. in from nineteen to twenty-seven hours. The animals were in such a weakened condition from the general effect of the toxin, that physiological studies were impossible. The powerful action of the toxin was shown by the occurrence of excessive vomiting, bloody diarrhea, muscular weakness, and, occasionally, convulsions. At autopsy a

general hemorrhagic condition, involving the serous and mucous membranes, was present; acute splenic tumor, and cloudy swelling of the heart, liver, and kidney were constant lesions; and occasionally hemorrhages in the kidney were found. The urine after ten to twelve hours contained traces of albumin and, later, red corpuscles; anuria was not infrequent.

A striking feature of the action of diphtheria toxin and one that has been emphasized by Schlayer and Hedinger in their work on rabbits, is, after a preceding period of apparently perfect health, the sudden onset of symptoms. The latent period in our experiments varied from five to twelve hours and was followed abruptly by severe symptoms of prostration, and death after a comparatively short period. That the toxin produced very slight changes in the vascular system almost immediately which can be demonstrated by physiological methods, is shown in the following experiments in which large amounts of toxin were injected intravenously.

TABLE I.

Normal Dog.

Dog, weight 8,940 grams, ether anesthesia.

	Time.	Blood pressure in millimeters mercury.	Kidney volume* in millimeters.
Initial reading	3.15	142	0
3. c.c. salt solution	3.16	142	0
0.1 c.c. diphtheria toxin	3.18	136	− 1
0.3 c.c. diphtheria toxin	3.23	128	− 3
3. c.c. diphtheria toxin	3.28	138	−11
3. c.c. diphtheria toxin	3.35	136	− 4
Final reading	3.45	134	− 5

*The horizontal level of the writing lever at the beginning of the experiment is taken as normal kidney volume for the conditions obtaining. Increase or decrease in volume, as the case may be, is indicated in millimeters preceded by a plus or minus sign. The change in level was measured on the tracing with the base line as a constant factor.

This evidence of slight immediate effect of the toxin, coupled with the observations on the severe conditions developing after twelve to fifteen hours, led to the use of small doses which would prolong the period preceding marked vascular disturbance. We have in this way been able to delay the appearance of the late lesion for twenty-

four hours, but never for forty-eight hours. To bring about this delay, doses as small as 0.0015 to 0.003 cubic centimeter of toxin per kilo. of body weight have been necessary. The early stage, which is essentially a tubular nephritis, is illustrated in the following experiment.

TABLE II.

Early Diphtheria Nephritis.

Dog, weight 8,000 grams, ether anesthesia. Received 0.003 cubic centimeter diphtheria toxin per kilo. subcutaneously twenty-two hours before test began. Urine showed trace of albumin.

	Time.	Blood pressure in millimeters mercury.	Kidney volume in millimeters.	Urine drops in 5 minutes.
Initial reading.....	3.15	102	0	5
Adrenalin.....	3.20	144	−25	—
	3.21–3.26	94	− 5	6
Caffein.....	3.30	—	—	—
	3.39–3.44	130	+21	25
Salt solution.....	3.45	—	—	—
	3.50–3.55	130	+19	72
Dog urine.....	3.56	78	−60	—

This experiment, except for a somewhat unusual increase of blood pressure after the injection of caffein, shows results typical of the reactions in early tubular nephritis and varying but slightly from those of the normal kidney. Similar results were obtained at the end of twenty-four hours in other dogs receiving approximately the same dose, but when the dose was raised to 0.025 cubic centimeter per kilo. the vascular reactions were, after the same period, less marked and diuresis less active. Even with smaller doses it was impossible to carry the animals through the second twenty-four hours without evidence of serious vascular disturbance. This is shown in the following experiment.

If this record be compared with that of table II it will be seen that the power of the renal vessels to contract under the influence of adrenalin is unaltered, but that the power to dilate under the influence of caffein and salt solution is lost; at the same time diuresis is, after the administration of caffein, unaltered and after salt solution is somewhat less than in the earlier stage of nephritis. This record exhibits apparently the transition from a renal lesion of the tubular type to one of the severe vascular type. So rapidly does the

TABLE III.

Diphtheria Nephritis, after Forty-eight Hours.

Dog, weight 7,980 grams, ether anesthesia. Received 0.0026 cubic centimeter diphtheria toxin per kilo. subcutaneously, 48 hours before testing. Urine contained albumin and casts.

	Time.	Blood pressure in millimeters mercury.	Kidney volume in millimeters.	Urine drops in 5 minutes.
Initial reading.....	3.45	86	0	2
Adrenalin.....	3.47	122	−31	—
	3.54–3.59	88	+ 2	3
Caffein.....	4.00	—	—	—
	4.05–4.10	110	0	24
Salt solution	4.11	—	—	—
	4.13–4.17	108	+3	45

latter come on that we have been able to secure only this one observation showing the transitional stage. The usual effect at eighteen to twenty-four hours after large doses, or at thirty-six to forty-eight hours after small doses, is given in the next experiment.

TABLE IV.

Severe Diphtheria Nephritis.

Dog, weight 5,430 grams, ether anesthesia. Received 0.09 cubic centimeter diphtheria toxin per kilo., twenty-two hours before the test. Fifteen hours after injection the dog vomited and developed diarrhea; at twenty-one hours he was much prostrated. At fifteen hours urine contained albumin. Anuria noted during the last seven hours.

	Time.	Blood pressure in millimeters mercury.	Kidney volume in millimeters.	Urine drops in 5 minutes.
Initial reading.....	3.00	20	0	0
Adrenalin.....	3.05	32	0	0
	3.14	20	0	0
Salt solution.....	3.15	—	—	—
	3.18–3.28	36	+7	0
	3.40	36	+4	0
Caffein.....	3.41	—	—	—
	3.45–3.57	28	+2	0

This experiment appears to illustrate the severe cardiac and vascular disturbances attending profound intoxication with diphtheria toxin; conditions which are the result of the general effect of the toxin and which render impossible conclusions concerning its late local or specific action on the kidney.

The histological study of sections from the kidneys of this series

shows the presence of both tubular and vascular lesions. The latter are the more striking in that in the severer lesions almost every glomerular tuft is congested and surrounded by a greatly distended capsular space filled with serum and fibrin. The tubules generally are filled with densely packed coagulated material and their epithelium is granular and vacuolated, while that of the convoluted tubules is frequently completely disintegrated. Hyaline and granular casts are fairly abundant. In the case of the milder lesions the evidence of epithelial injury is more striking than that of vascular injury, while the kidneys of those animals which received very small doses of toxin show no exudative lesions whatever.

Both the anatomical and physiological studies indicate, therefore, that during the early period after the administration of diphtheria toxin, the condition in the kidney is analogous to that described by Schlayer and Hedinger as a tubular nephritis; yet the severity of the disturbance of the later period, as shown by physiological methods, gives the lesion a prominent place in that group of nephritides in which vascular injury is the most prominent feature.

THE EFFECT OF TUBERCULIN AND MALLEIN.

We used these products in the hope of obtaining, by the use of bacterial poisons, vascular lesions of less severity than those caused by diphtheria toxin. In this we were disappointed, as the lesions were essentially those of a mild tubular nephritis. Both products, in concentrated form, were obtained through the kindness of Dr. Reichel of the Pennsylvania State Live Stock Board Laboratory. The tuberculin had been prepared from bovine tubercle bacilli. When injected subcutaneously and intraperitoneally in doses varying from three to twenty cubic centimeters and repeated sometimes on two or three successive days, these substances failed to produce any general clinical effect. The urine of all animals, however, showed traces of albumin which was sometimes in large enough quantity to yield a slight flocculent precipitate. Physiological studies of the kidney failed to show results differing from those shown by an early tubular nephritis. Somewhat exaggerated vascular reactions and free diuresis occurred such as are seen in the early slight nephritis due to uranium and chromium salts. Sometimes the vari-

ation was so slight as to suggest merely an exaggeration of the reactions of the normal kidney; but the presence of albumin in the urine and the histological evidence of extensive granular and vacuolar degeneration of the tubular epithelium pointed conclusively to a tubular nephritis of slight severity. These effects, when contrasted with the severe vascular disturbance caused by diphtheria toxin, are of interest as demonstrating the occurrence of a renal lesion of bacterial origin essentially epithelial in character.

THE EFFECT OF NEPHROTOXIC IMMUNE SERUM.

The lesion caused in the dog's kidney by the so called nephrotoxic immune serum[3] consists of a form of renal injury more closely analogous etiologically to certain forms of nephritis in man than those produced by other experimental procedures, and it is therefore highly worth study by physiological methods. A serum prepared by the repeated injection into the rabbit of dog kidney cells, freed from blood, has been utilized for this purpose. Four dogs received serum from three treated rabbits, in doses of two cubic centimeters per kilo. of body weight. Physiological tests were made on the second, third and fourth days after the injection, and at a time when the urine of the respective animals contained abundant casts and an amount of coagulable protein varying from three fourths to three and one half grams by the Esbach method. The tests gave results characteristic of tubular nephritis; that is, exaggerated vascular reactions and excessive diuresis.

Histologically the kidneys of these animals showed lesions of the tubular epithelium with fairly abundant cast formation, and occasionally a slight accumulation of serum in the glomerular capsule. Physiological study, therefore, supports the conception based on histological examination, that this lesion consists essentially of a tubular nephritis. The slight changes in the glomerular capsule, however, point to some vascular injury, presumably of such a nature or of so slight a degree as not to be recognized by physiological methods.

[3] Pearce, R. M., An Experimental Study of Nephrotoxins, *Univ. Penn. Med. Bull.*, 1903, xvi, 217.

THE EFFECT OF HEMOLYTIC IMMUNE SERUM.

The most characteristic lesion in the kidney of an animal receiving a hemolytic serum is, aside from evidence of the destruction of the red corpuscles and of epithelial degeneration, the occurrence of an exudate, composed chiefly of serum, in the glomerular capsule. As this effect is evidence of injury to the capillary loops of the glomerular tufts, the lesion would appear particularly suitable for the study of vascular reactions. A hemolytic immune serum was prepared by repeatedly injecting rabbits with the red blood corpuscles of the dog, and the serum so obtained was then injected intraperitoneally into dogs, in amounts sufficient to cause hemoglobinuria. The physiological tests were performed at two periods: (*a*) the height of the elimination of hemoglobin; and (*b*) shortly after it had disappeared from the urine.

The results of these tests, contrary to the expectation based on the anatomical evidence of extensive glomerular injury, were not such as to indicate the existence of vascular insufficiency, but were those indicative of the occurrence of a so called tubular nephritis. Exaggerated reactions to caffein and salt solution, and marked diuresis instead of the expected decreased dilatation and diminished diuresis were obtained. The results indicate that injury to the vessels of the kidney sufficient to permit exudation is not necessarily, physiologically speaking, that leading to reactions of vascular nephritis; but unlike the lesions caused by arsenic and diphtheria toxin, cannot be recognized by the physiological procedures in this investigation. The injury produced by arsenic is apparently one interfering with the mechanism which causes the vessels to contract and dilate, while the lesion following hemoglobinuria is, apparently, one affecting only the endothelial membrane which controls the passage of fluid. It is evident, therefore, that we may have, as the lesion indicates, anatomical evidence of vascular injury which cannot be recognized by the physiological methods employed for the purpose by Schlayer and Hedinger; while, on the other hand, we may have, in arsenical nephritis, physiological evidence of serious vascular nephritis in the absence of demonstrable evidence of anatomical changes.

CONCLUSIONS.

The physiological study of experimental nephritis caused by poisons of bacterial origin demonstrates that the poisons may produce types of nephritis in which either vascular or tubular changes predominate. Diphtheria toxin produces a nephritis which in its late stage is of the vascular type, but in its early stage is distinctly tubular. Tuberculin and mallein uniformly cause lesions of the tubular type, which do not pass into the vascular type.

Nephrotoxic and hemolytic immune sera cause changes in the kidneys which by physiological methods of observation present no evidence of vascular injury, but which are anatomically characterized by exudative glomerular lesions of moderate severity. This discrepancy between the results of anatomical and physiological study indicates that a lesion of the membrane controlling the passage of fluids may occur without alteration in the power of the vessels to contract and dilate. This fact is shown clearly by the lesion caused by hemolytic immune serum, which is in sharp contrast to the lesion caused by diphtheria toxin, since the latter substance not only alters the permeability of the membranes but also influences markedly the power of the vessels to contract and dilate. It is necessary therefore if the term "vascular" is used in its broadest sense, to recognize three types of vascular nephritis: (1) one in which little or no anatomical evidence of vascular injury is found, but in which physiological methods show profound vascular changes, as in arsenical nephritis; (2) one in which anatomical evidence of vascular (exudative) injury is prominent, but in which the physiological tests are negative, as in nephritis caused by a hemolytic immune serum; and (3) one in which both anatomical and physiological changes are prominent, as in diphtheria toxin nephritis.

THE QUESTION OF ATHREPSIA.*

By PROF. H. APOLANT, M.D.

(From the Königl. Institut für experimentelle Therapie, Frankfurt a/M, Director, Wirkl. Geh. Rat. Prof. Dr. P. Ehrlich.)

In a recent number of this Journal[1] an article by I. Levin and M. J. Sittenfield, "Studies on Immunity in Cancers of the White Rat," appeared, which, as is indicated by its sub-title, "The Significance of Athrepsia," presented a criticism of the athrepsia hypothesis of cancer immunity as set forth by Ehrlich. In consideration of the lack of clearness concerning the nature and significance of athrepsia which is there indicated, the deductions drawn by the authors of this article should not remain unchallenged. The authors are in error in an early sentence, namely: "The most popular and most generally accepted theory of cancer immunity is the one advanced by Ehrlich and is commonly designated the athrepsia hypothesis." With much more justice it might be maintained that the athrepsia of Ehrlich is the most misunderstood and precisely on this account the most frequently misrepresented of all theories of cancer immunity. The work of Drs. Levin and Sittenfield offers a particularly striking proof of this.

On page 512 occurs the following sentence: "When the organism of the host is immunized by treatment with a cell emulsion, these cells bind the specific food and consequently the cancer cells inoculated subsequently do not find the necessary nourishment and die. Immunity, then, to cancer growth consists in the lack of food athrepsia." How the authors arrive at this remarkable conclusion is incomprehensible; certainly it would be difficult to find verifica-

* On account of the importance of the questions involved as well as the eminence of the Institute from which this paper emanates, the Editor of the *Journal of Experimental Medicine* is departing, in this instance, from the policy of the *Journal,* which is to decline to publish articles purely critical of previous experimental papers appearing in its columns. Received for publication, July 12, 1911.

[1] Levin and Sittenfield, *Jour. Exper. Med.*, 1911, xiii, 511.

tion for it in the reports of the work which have been issued from the Institute at Frankfurt. They have simply confused the actively produced tumor immunity with that of athrepsia, and appear not to be aware that Ehrlich has always separated the two immunities sharply from one another. In one of his latest publications[2] on the subject he says, "The question naturally arises as to the foundation of the acquired immunity. Were it dependent on the formation of specific antibodies,—a possibility to be first considered,— then the production of passive immunization with the blood of immune animals would inevitably follow. Up to the present time only a few data on this point have been under discussion,—by Jensen[3] among others; and the figures, particularly those published by Clowes and Baeslack,[4] are not convincing. Since these authors did not work with tumors of maximum virulence, the possibility remains that the quantity of antibodies formed was insufficient for demonstration with certainty by the test of passive immunization. At any rate we should mention that in the various centres for cancer research, (the Rockefeller Institute, London, Frankfurt), attempts at the demonstration of such antibodies, either in experiments with animals, or *in vitro,* have turned out on the whole negatively. Although it is not yet possible completely to rule out antibodies, nevertheless still further experiments are necessary in order to gain a clear insight into this question. For various reasons, however, I consider it inadmissible to bring forward, on the basis of present knowledge, and without anything further by way of positive fact, the hypothesis that the immunity directed against mouse cells rests on isolysins." Thus not a word is uttered concerning athrepsia. On the other hand, he continues further on, "So much, gentlemen, concerning active immunization. You will permit me at this point to enter somewhat more fully into the question of another form of immunity, to the assertion of which I was led years ago by reason of many peculiarities of mouse tumors; namely, immunity by athrepsia." Here, therefore, immunity by athrepsia is brought into sharp contrast with active immunity, a fact which seems wholly to have escaped Drs. Levin and Sittenfield.

[2] *Verhandl. d. deutsch. path. Gesellsch.,* 1908, xii, 17.
[3] *Centralbl. f. Bakt., Orig.,* 1903, xxxiv, 28; 122.
[4] *Med. News,* 1905, lxxxvii, 968.

The remarks of these authors on the subject of panimmunity suffer in the same way. They begin this division of their paper with the sentence: "Ehrlich did not consider the phenomenon of panimmunity a direct proof of athrepsia," and continue immediately, "but there must undoubtedly be a certain relation between the general non-specific character of the former and athrepsia, which is merely a phase in intracellular nutrition. Indeed, if athrepsia is to have a general application it must be capable of explaining the conditions of panimmunity against the different tumors used in this research, and it may be stated that in a general way the fact of panimmunity was found to be correct for the majority of cases." It may be replied, that athrepsia does not have a general application, and that it has never been brought by Ehrlich into relation with panimmunity. Hence the authors' method of treatment leads to a misinterpretation of the theory of athrepsia, which thus becomes the object of criticism that could not apply to the theory correctly formulated. This error is to be deplored.

The case is different with the further divisions of the article, in which the authors raise objections to athreptic immunity, where according to Ehrlich they surely have weight, first in regard to the so called zigzag transplantations between mouse and rat. But here again we find a sentence which shows that they have again fallen into error: "On the basis of this supposition, Ehrlich created the general theory that whenever cancer cells fail to proliferate, it means that they fail to obtain the food x, either because the normal body cells have greater avidity for this food than the cancer cells, or else the cells with which the animals were immunized anchored all the specific food and the cancer cells inoculated subsequently could not obtain it."

The authors adduce against Ehrlich's explanation that the inoculation-yield by zigzag inoculations is considerably less than in the control inoculations in the same species, and hold that this fact is to be explained not by lack of specific food stuff, but merely by the presence of directly injurious substances. That the yield on inoculation in a foreign species is in the first place not so good as within the same species would appear to be not remarkable, since the conditions for proliferation in the first instance are naturally less favorable.

But if these zigzag inoculations are continued, which the authors in question seem not to have done, the results improve, and above all, the growth of the tumors, if they grow at all, shows no retardation. This is irreconcilable with the conception of injurious substances. In this case it is not the unsuccessful but the successful inoculations which point to the true explanation. If rats possessed preformed antibodies against mouse tumor cells, the latter would in no single instance grow in the rat to a transplantable tumor, which, even according to Levin and Sittenfield, is reinoculable into the mouse in twenty-five per cent. of the animals.

Moreover these authors do not allow to stand unqualified the second form of athrepsia postulated by Ehrlich, which is observed in the same animal after double inoculation. Neither with the Flexner-Jobling rat carcinoma, nor with the Ehrlich rat sarcoma did they fail to obtain takes of the second tumor graft. It is, however, noteworthy that the rat carcinomata possess far too slight a growth for the demonstration of this form of athrepsia. But even in the case of the considerably more virulent rat sarcoma the athreptic influence of the first tumor could be observed, primarily in the retarded growth of the subsequently inoculated tumor. Whether Drs. Levin and Sittenfield considered these facts and took into account the weight of the tumors which Ehrlich on his side has required, is not apparent.

Again, they deny a general value to my results with the inoculation of mixed tumors,—results which Ehrlich has explained in connection with athrepsia. I had been able to show, that by the inoculation of mixed but unlike virulent tumors only the more virulent component comes to development, and that, therefore, a true mixed tumor is only obtained when the stronger power of proliferation of the more virulent tumor is artificially set aside. Levin and Sittenfield corroborate my results for the mixture of mouse carcinoma and sarcoma, but not for the corresponding rat tumors, also with divergent degrees of virulence. From their very short and summary data, to which no protocols at all are added, it does not appear whether in the development of the two components no quantitative differences were present. For various reasons, not to be further dealt with in this place, the results of such inoculation of mixed

emulsions naturally vary within certain limits, so that it is permissible to draw positive conclusions only after very numerous experiments. Drs. Levin and Sittenfield do not state the number of inoculations made by them.

Finally, the authors concede that conditions of nutrition for the growth of the tumor grafts enter into the question, but the proof of this adduced,—namely, the stroma reaction postulated by Russell,[5] —shows plainly that these conceded nutritive conditions have nothing to do with the athrepsia of Ehrlich. It should also be mentioned that the value of Russell's investigations has become exceedingly problematic by reason of the subsequent very exact repetitions carried out by Goldmann. It should be further noted that Drs. Levin and Sittenfield misinterpret some of the facts and fail entirely to mention others which demonstrate the existence of athrepsia. The former applies to their presentation of the relation of tumor growth to pregnancy. They refer on the one hand to Haaland,[6] who first observed the resistance of gravid animals to tumor inoculation, and on the other to Herzog,[7] who described a more rapid growth of the tumor in the course of pregnancy. They conclude immediately from these observations that pregnancy simply inhibits the take of an inoculated graft, but that it stimulates the growing tumor to stronger proliferation. This conclusion is entirely arbitrary, and rests on a mistaken citation. For Herzog speaks not of tumor-bearing animals, which became pregnant, but of inoculation of pregnant animals. On the other hand, Cuénot and Mercier found that the beginning of tumor growth can be temporarily inhibited by the onset of pregnancy, and by lactation, only to proceed thereafter in a normal manner. The apparent inconsistencies in the observed relations between pregnancy and tumor growth are probably explained, as Fichera has already observed, by the fact that, if numerous embryos are present, the specific foodstuffs, which are often the same for embryos and for tumor cells, are almost wholly demanded by the former, but that, on the other hand, when only a few embryos are present, the production of the

[5] *Scientific Reports of the Imperial Cancer Research Fund,* 1908, No. 3, 341.
[6] *Berl. klin. Wchnschr.,* 1907, xliv, 713.
[7] *Jour. Med. Research,* 1902, viii, 74.

specific food stuffs, increased by pregnancy, comes to benefit the tumor cells also. It is reasonable and entirely consistent with the principle of athrepsia that occasionally an already strongly growing tumor should draw still more strongly on the food stuffs abundantly produced at the advent of pregnancy, and so grow still more quickly.

Drs. Levin and Sittenfield do not mention at all the experiments of Pierre Marie and Clunet, which are in direct verification of the theory of athrepsia. Ehrlich, in 1905, had already advanced the supposition that the rare occurrence of metastases in animals inoculated with very virulent tumors, in contrast to the relative frequency of secondary nodules in animals having slowly growing spontaneous tumors, was due to the fact that in the former case the specific food stuffs of the embolized cells were appropriated by the original tumor. Marie and Clunet have recently found that after operative removal of rapidly growing tumors, metastases occur in fifty per cent. of the animals; and they are of the opinion that the facts are best explained by Ehrlich's theory of athrepsia.

In conclusion we desire to urge that a theory as well founded as that of athreptic immunity be not subjected to discredit without the presentation of all the facts bearing upon its various sides, with a degree of fullness and clearness of interpretation that will permit of a convincing conclusion.

NERVE FIBRILLÆ IN THE PULMONARY ARTERY OF THE DOG.*

By HOWARD T. KARSNER, M.D.

(From the McManes Laboratory of Pathology, University of Pennsylvania, Philadelphia.)

PLATE 29.

The work reported in this paper has been conducted intermittently during the past two years in conjunction with studies of Dr. Horatio C. Wood, Jr., on the physiology of the arterial nerves of the pulmonary circuit.

The nerve supply of the lungs comes from the small anterior pulmonary plexus and the larger posterior pulmonary plexus, these plexuses being formed by branches from the vagi and sympathetic nerves. Branches from the plexuses accompany the lung roots into the organs and are there distributed to the bronchi and blood-vessels. The coarser distribution of the nerves is easily demonstrable; it was pointed out in man by Stirling in 1876, and since then has been reported by various observers in many of the higher mammalia.

The problem of the finer distribution, however, has been the subject of less satisfactory research. Arnold claimed that there were three nerve plexuses in the arterial walls of the general circulatory system, an outer or adventitial network loosely arranged and often containing medullated fibers, a second network loosely arranged in the muscle layer and made up of fine but regularly outlined fibrils, and a third network finely arranged and made up of varicose non-medullated threads which end in nodular or knob-like thickenings. This description was supported by numerous writers, among the most recent being Michailow, who used methylene blue dissolved in the Ringer-Locke solution. Barbieri, however, having worked with the arteries of dogs, thinks that Arnold's plan is too diagrammatic

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for actual demonstration. My own work, although limited to the pulmonary artery, leads me to accept the latter view.

As to the special demonstration of nerve fibrils in the pulmonary arteries, little has been done, the majority of workers contenting themselves with more easily accessible organs. Retzius, however, noted their presence in a human embryo, and Berkley, using the rapid Golgi method, was very successful in demonstrating the presence of nerve fibrils in the pulmonary artery of rabbits, mice, and gray and white rats. He found that the "plexuses that accompany arteries are not so well developed as those about the bronchial tubes; still traces of them are not infrequently met with in the form of single fibres and portions of anastomosing networks following the direction of the artery, and here and there an end knob may be found, . . . seemingly near or between the smooth muscular cells of the middle coat." He states further that "nerve fibres in sparse number may also be observed in the walls of the larger veins."

Personally I have tried several modifications both of the silver stains and of the gold stains, but I found them unsatisfactory because of the presence of a very rich elastica in the arterial walls in the lung, a feature that I have demonstrated by the use of the Weigert elastica stain in paraffin sections. The Bielschowsky method as modified by Schütz also was unsatisfactory. For this reason, I turned my attention to the use of methylene blue. The method of Dogiel was used on frozen sections without satisfactory results. Bethe's modification of the Ehrlich intra-vital method failed. Leontowitsch's method seemed to offer more promise, but before I was able to work it out I read of the method of Huber, and after modifying it so as to meet the conditions of my own problem, used it with success. His work was with the cerebral arteries of dogs, rabbits, and cats. He injected into the carotid artery a 1 per cent. solution of methylene blue in normal salt solution, in sufficient amounts to tinge the ear and eye, and, after thirty or forty minutes, made his examination. Inasmuch as I wished to stain the arteries of the lung, I injected the solution into the jugular vein, which was exposed under ether anesthesia, until the skin of the belly was distinctly blue. The animal was permitted to live for from fifteen to thirty minutes, often requiring artificial respiration. I used a 1 per

cent. solution of methylene blue, usually employed for making up the ordinary stains, mixed in an 0.85 per cent. sodium chlorid solution. I tried similar solutions of medically pure methylene blue, but the staining results were unsatisfactory. After fifteen to thirty minutes, the dogs were killed by rapid injection of about 100 cubic centimeters of the methylene blue solution. One half hour later, the lungs and heart were removed *en masse,* and starting at the right ventricle where the identification of the pulmonary artery was readily made, I found the larger part of the pulmonary arterial system filled with paraffin (the melting point of the paraffin being 45° C. so as not to "cook" the tissues). The finer branches (those with a calibre of from 2 to 4 millimeters) were dissected out, freed from surrounding tissues, and permitted to oxidize for from ten to thirty minutes. They were then frozen in 0.7 per cent. ammonium molybdate solution and cut on the flat surface so as to strike the media of the vessels. The sections, still in the molybdate solution, were mounted on slides, oxydized further, and covered with glycerin and a cover slip. Low power examination of the walls, before being frozen and sectioned, often showed a coarse adventitial network made up probably of nerves. Examination of the histologic preparation with a one twelfth oil immersion lens showed the rich mass of unstained elastica and smooth muscle, in the midst of which occasional networks such as those shown in the drawings were found. The fibrils were fine and showed distinct beading or varicosities similar to those observed with this stain by all the more recent workers. This feature is shown in figures 1 and 2 in the drawing. In only a few of the sections was I able to demonstrate end bulbs, but in these sections they seemed distinctive. (See figures 1 and 4.) Possibly because of my insufficient experience with the technique, none of the bulbs showed the exceedingly rich and fine network described and depicted by Hofmann. This fine end network, however, has not been demonstrated by the majority of writers, who seem to have found, as I have, simply a terminal bulb.

In the drawing, some of the bulbs seem to be placed in the midst of the network, as is the case in figures 1, 3, and 4; but as the sections were necessarily thick, I am of the opinion that they were simply end bulbs situated above the fibrils. In the case of figure

3 in the drawing, there is to be seen what apparently is an intercalated cell, but closer study led me to think that it was an artefact or a superimposed end bulb.

The study was controlled by injecting the solution of methylene blue into the dorsal lymph sac of a frog and examining frozen sections of the heart. The cells and fine fibrillæ in the ganglia made me additionally sure of my findings in the sections described above.

From my study, I feel justified in the conclusion that the media of the pulmonary artery of the dog possesses a nerve supply capable of histologic demonstration.

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EXPLANATION OF PLATE 29.

A camera lucida drawing with a Spencer 1/12 inch oil immersion lens and Spencer no. 2 ocular.

All figures show the characteristic beaded appearance. Figures 1, 3 and 4 show end bulbs. In figure 3, intercalated cells are seen, which probably are over-riding end bulbs. Figure 2 shows the heaping up of deeply staining material at the points of branching of the network—a fairly common feature.

STUDIES OF THE PULMONARY CIRCULATION.*

By HORATIO C. WOOD, Jr., M.D.

(From the Department of Pharmacology and Therapeutics in the Medico-Chirurgical College, Philadelphia.)

Plates 30 to 32.

The question of whether or not the blood-vessels of the pulmonary circulation are under the control of the central nervous system has been a subject for difference of opinion for many years. Several years ago I published (1) the results of some experiments bearing upon this problem, which led me to a belief in the existence of a vasomotor supply to the lesser circulation. Since then I have carried on further investigation of the pulmonary circulation, with especial reference, however, to the effects of drugs upon it. The results which I have obtained in this second series of experiments have strengthened the conviction obtained from my first work on the subject.

Before proceeding, however, with the report of my own experiments, I wish to call attention to the histological research of Dr. Karsner, published simultaneously with this paper. Dr. Karsner, who interested himself in this problem at my suggestion, has succeeded in demonstrating, by a method of *intra vitam* staining with methylene blue, the presence of nerve filaments in the muscular coat of the pulmonary artery. This evidence seems to me of extreme importance. Brodie and Dixon brought forward as one of their arguments against the probability of a pulmonary vasomotor system, that they were unable to find, microscopically, any nerves in these vessels; but as they themselves recognized, such negative evidence is not of great weight, because the failure to find nerve filaments in the pulmonary arteries does not disprove their existence; on the other hand, a positive result is of much value. It is improbable that

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nerves can exist without performing some function, and the most natural, and, indeed, the only natural interpretation of nerve filaments in the arterial muscles is that they are concerned in regulating the contraction of these muscles. It would seem to me, therefore, that the results of Dr. Karsner's investigations are almost sufficient of themselves to settle definitely the question.

I have studied the action of a number of agents upon the pulmonary circulation and have found the following to exercise an influence well enough marked to merit further discussion: asphyxiation, epinephrin, the nitrites, ergot, digitalis, and ether. I shall consider each of these separately.

Asphyxia.—That asphyxiation causes a rise of the pressure in the pulmonary artery, as in the general circulation, was demonstrated as far back as 1882, by Openchowski (2). He believed, however, that the increased pressure was not brought about by a constriction of the pulmonary arteries, but by the constriction of the arteries of the aortic system preventing the left ventricle from completely emptying itself, so that the blood was dammed back in the left auricle and pulmonary veins, thus increasing the resistance in front of the right heart. This view, however, is not tenable, because in the first place the pressure in the pulmonary artery may begin to ascend at a time when that in the carotid is increased as little as ten millimeters, and there is no adequate reason to suppose that such slight augmentation in the resistance is able to overcome the reserve power of the ventricular muscle. Moreover, I have found that by mechanically obstructing the passage of blood from the heart, through compression of the thoracic aorta, it is possible to cause an increase of from forty to fifty millimeters in the carotid pressure with practically no effect on the pressure in the vessels of the lungs. Still more conclusive is the fact that if the rise in the systemic pressure is prevented by destruction of the vasomotor mechanism, as by cutting the spinal cord, asphyxiation will still cause an elevation of the pulmonary pressure.

A striking fact, which has been commented on by several observers, is the slowness with which the blood pressure rises in the pulmonary artery under asphyxiation. It was this delay which led Openchowski to believe that the rise was due to the stagnation of the

blood in the left auricle; he believed that the difference in the time between the beginning of the rise of the pressure in the aorta and in the pulmonary artery was needed to increase the resistance in the aortic system sufficiently to dam the blood back upon the lungs. But this is evidently not the true explanation, because of the insignificance of the rise in the aorta during this time, although the length of time which intervenes is considerable. In Openchowski's experiments, the pressure began to rise in the carotid artery on the average within ten seconds after shutting off the air, but in the pulmonary artery not until a full minute had elapsed. In my own experiments, the average length of time required before the beginning of the rise in the carotid artery was sixteen seconds, and in the pulmonary artery forty-four seconds (figure 1).

If the time necessary to produce the reflex in the pulmonary artery is not to be explained by the congestion theory of Openchowski, to what cause can it be attributed?

In the discussion of the question of the existence of a vasomotor supply to the lungs, it has always been taken for granted that if there were pulmonary vasomotor centers, these, like those of the general circulation, were to be found in the medulla oblongata. It is well known that there are vasomotor centers, more or less independent of the dominating centers in the medulla, located throughout the thoracic portion of the spinal cord, and that after section of the cord in the cervical region, these centers are, under certain conditions, sufficiently powerful to cause constriction of the blood vessels on asphyxiation. The characteristic difference between the spinal vasomotor centers and those in the medulla is that not only are they more feeble, but also slower in their response to the accumulation of carbonic acid. The thought, therefore, suggested itself: may it not be that the delay in the response of the pulmonary circulation is due to the fact that the vasomotor supply for the lungs comes solely from the spinal cord and not from the medulla?

Bradford and Dean have found that after section of the spinal cord above the third dorsal vertebra the rise of pressure in the pulmonary artery on electrical irritation of the medulla is much less than if the cord has been cut at the seventh dorsal vertebra, although in neither case was there any increase in the carotid pressure. This,

of course, merely indicates that the nerves carrying impulses to the pulmonary arteries leave the cord below the third cervical vertebra. If the theory which I have suggested is correct, then the destruction of the medullary vasomotor centers should not prevent the rise in pulmonary pressure. For the purpose of excluding the centers in the medulla, I have used the method described by Asher and myself (4) of injecting paraffin into the cranial end of the carotid artery. In the three experiments in which this method has been carried out, I have obtained rises in the pulmonary artery of from 50 to 100 per cent., accompanied either with no change or else a fall in the carotid blood pressure (table I).

TABLE I.

Time.	Carotid pressure.	Pulmonary pressure.	Remarks.
Beginning	85	8	Beginning of asphyxia.
0 min. 30 sec.	125	9	
0 min. 50 sec.	167	12	End of asphyxia.
1 min. 30 sec.	126	9	
2 min. 30 sec.	110	8	Injection of paraffin into carotid artery.
2 min. 40 sec.	133	10	
4 min. 0 sec.	200	14	
4 min. 25 sec.	230	28	
5 min. 30 sec.	138	9	
9 min. 30 sec.	117	8	Beginning of asphyxia.
10 min. 0 sec.	108	8	
10 min. 10 sec.	110	11	
10 min. 20 sec.	95	16	End of asphyxia.
10 min. 25 sec.	98	17	
11 min. 0 sec.	109	9	
14 min. 0 sec.	106	8	Beginning of asphyxia.
14 min. 25 sec.	98	13	
14 min. 30 sec.	90	18	
14 min. 50 sec.	83	20	
15 min. 0 sec.	Clot	20	End of asphyxia.
16 min. 0 sec.	98	14	

Post mortem examination showed the entire circle of Willis and both vertebral arteries completely filled with paraffin.

Epinephrin.—As far as I have been able to find, Velich (3) was the first to demonstrate that there occurred, after the injection of the suprarenal extract, a marked rise of pressure in the pulmonary as well as in the carotid artery. This fact has been so abundantly confirmed that it needs no further remark (table 2).

Brodie and Dixon (5) insist, however, that this rise does not mean a constriction of the pulmonary arteries; they base their conclusions

TABLE II.

Time.	Carotid pressure.	Pulmonary pressure.	Remarks.
Beginning	89	13	Both vagi have been cut. Injection of 1.5 c.c. adrenalin solution.
0 min. 30 sec.	96	16	
1 min. 0 sec.	135	19	
2 min. 0 sec.	279	24	
10 min. 0 sec.	174	18	Clot in interval.
20 min. 0 sec.	85	16	Injection of 1.5 c.c. adrenalin solution.
20 min. 30 sec.	85	19	
21 min. 0 sec.	200 (?)	21	
22 min. 0 sec.	244	26	
25 min. 0 sec.	224	25	

on the results obtained by perfusing solutions of the drug through isolated lungs. Their method was to separate the lungs as rapidly as possible from the body, and force through them defibrinated blood under constant pressure, measuring the amount of fluid which came out through the pulmonary vein in a given period of time. They found that, as a rule, not only was the passage of blood through the lungs not delayed, but usually even hastened, which they interpreted to mean that there was a dilatation rather than a constriction of the pulmonary artery; and as this drug is conceded to be one of our most reliable stimulants to the peripheral nervous apparatus of the circulation, they concluded that there could be no nerve supply in the pulmonary artery. Brodie, however, in an address delivered before the Royal College of Surgeons (6), describing their results, said: "Only in one instance have we obtained constriction." Further on in the same address, he mentions some experiments which were made by measuring the lung volume, which are not included in their formal communication. In these it was found that: "The effect of suprarenal extract upon the blood volume of the lung is to cause first a small diminution in the volume, soon followed by a marked increase which persists during, and for a short time after the rise in aortic pressure." It is difficult for me to understand how an increased output of the heart can cause a diminution in the blood volume of the lung. Is it not much more reasonable to suppose that there was a contraction of the pulmonary vessels which lasted until the increase in the pressure in the left heart caused by the extraordinary resistance in the aortic system became so great that the relatively feeble pulmonary muscles were unable to withstand the strain?

From the fact that in one instance they obtained evidence of what they deem a "marked constriction" of the pulmonary arteries by the suprarenal extract, it would seem highly probable that the failure to obtain vascular constriction in the other instances was due to some error in technique. This conclusion is borne out by the fact that both Plumier (7) and Wiggers (8) obtained positive results, in each instance the work of Brodie and Dixon being known. Wiggers attributes the failure of the English investigators to obtain retardation of the flow to differences in viscosity, due to the fact that the epinephrin was dissolved in a fluid not homogeneous with that used for perfusion.

It is of interest in this connection to note the time relation between the elevation of blood pressure in the carotid and pulmonary arteries as brought about by epinephrin, especially in comparison with the effects of asphyxia. The elevation of the blood pressure is brought on by this agent with almost instantaneous promptness in the pulmonary as well as the general circulation; in fact, if anything, the rise in the pulmonary pressure is the more prompt. For instance, in the experiment represented in table II, thirty seconds after the injection of the first dose of epinephrin there was a rise of three millimeters, or 23 per cent. in the pulmonary pressure; while in the same period in the carotid artery, the rise was less than 8 per cent. At the end of the first minute, however, the carotid pressure having risen about 50 per cent., there is an abrupt change in the pulmonary curve, the pressure ascending much more rapidly and falling gradually more or less parallel with the fall in the carotid pressure. The same thing happened, but more distinctly, after the second injection, there being a marked break in the pulmonary curve when the carotid pressure had risen about 50 per cent. above its original height. The instantaneousness of the rise in the pulmonary pressure seems to me to preclude effectually any idea that the rise is due to a stagnation from the great resistance in front of the left heart.

Of course, cardiac changes are not positively excluded as the cause of the rise of pressure in this experiment, but taking all the facts together it seems to me that the most reasonable interpretation of all the results is that adrenalin caused a constriction of the

pulmonary as well as the general vessels, but under the influence of this powerful vasomotor stimulant the resistance offered by the constricted blood vessels was so great that the heart was unable to empty itself and that the blood was dammed back upon the comparatively feeble pulmonary artery, which then dilated passively, in much the same manner as may be seen at times in the vessels of the limb under powerful vasomotor stimulants.

This explanation of the succession of events is confirmed by the experiments of Brodie already quoted, in which the volume of the lung was at first diminished and secondarily increased, by the action of epinephrin, and also by the studies of Petitjean, who found that the drug produced a primary pallor of the lungs followed by a marked congestion.

Ergot.—The action of ergot on the two circulatory systems shows an interesting difference, although the meaning is obscure. The typical changes in the carotid pressure which follow an intravenous injection of an active preparation of ergot are a primary fall, followed in about a minute by a rise to a point markedly above the normal; the rise endures for a varying length of time according to the dose and condition of the animal, and then descends gradually to the original height, or sometimes slightly below. The second dose will cause the same phenomena, except that the rise is of shorter duration, and the subsequent fall carries the pressure nearly always below the normal. After subsequent injections the rises become progressively smaller and shorter, and there is a general tendency of the pressure constantly downwards.

The mode in which these changes are produced is not certainly known, but it is probable that the rise in pressure is due to a simultaneous action of the drug upon the heart muscle and upon the vascular muscles, with perhaps also some stimulation of the vasomotor centers. (Under the term "vascular muscles" I include the action not only upon the muscle tissue itself, but also upon the contained nervous elements in the walls of the artery.)

In the pulmonary circulation, the tendency of the pressure is always upward, even after enormously toxic doses. Both the primary fall, which follows immediately the injection of ergot, and the late fall in pressure, which occurs after the injection of repeated

doses, are absent in the pulmonary artery. As a result of the continual falling in the carotid pressure, and the ascending pressure in the pulmonary artery, the two curves approach more and more closely to each other until in one experiment the two needles actually interfered with each other (figure 2).

TABLE III.

Time.	Carotid Pressure.	Pulmonary Pressure.	Remarks.
Beginning	122	19	Injection of 1 c.c. fluid extract ergot.
0 min. 30 sec.	137	26	
1 min. 0 sec.	140	25	
3 min. 0 sec.	129	25	Injection of 1 c.c. fluid extract ergot.
3 min. 30 sec.	140	28	
7 min. 0 sec.	123	30	Injection of 1 c.c. fluid extract ergot.
8 min. 30 sec.	125	36	" " 1 c.c. fluid extract ergot.
9 min. 30 sec.	70	32	
10 min. 0 sec.	38	25	
10 min. 30 sec.	——	—	Needles interfere with each other.

Weight of dog, 12 kilos.

Three possible explanations of the difference of effects of ergot upon the two circulations suggest themselves: first,—that the large dose of ergot acts as a depressant to the left ventricle, but not to the right; second,—that there is a paralysis of the aortic vessels, but not of the pulmonary; and third,—that the fall in the general pressure is due to the diminished blood supply to the left heart on account of extreme vascular constriction in the lungs. It was suggested by Holmes (9), who was the first to bring forward adequate proof of the contraction of the general blood-vessels by ergot, that the primary fall in the pressure after the administration of this drug was due to spasm of the pulmonic capillaries. This theory, however, failed to receive general acceptance, but it is interesting to revive it in connection with the late effects of the drug upon the vessels in the lungs.

Ether.—Moderate doses of ether produce in the general blood pressure, ordinarily, but slight change, although there is usually a tendency towards lowering. In the pulmonary artery, on the other hand, ether produces a rise which, in some cases, has been extraordinarily great.

Although in one or two experiments I have tried introducing

ether vapors with the inspiratory air, the inaccuracy of the dosage and the technical difficulties seem to me to far outweigh any theoretical advantage over the intravenous administration. In order to insure the solution of ether in the blood, I have used for the injection a mixture of equal parts of ether, alcohol, and physiological salt solution. In passing, it may be said that the alcohol in this mixture can play no part in the effects, first, because the dose is too small, and second, because I have found that the action of alcohol upon the circulation in the lungs is as feeble and uncertain as it is upon the general circulation.

TABLE IV.

Time.	Carotid pressure.	Pulmonary pressure.	Remarks.
0 min. 5 sec. to 0 min. 30 sec.	123	26	Injection of 4 c.c. ether solution (33 per cent.).
0 min. 40 sec.	77	26	
1 min. 0 sec.	83	29	
2 min. 0 sec.	97	47	
3 min. 0 sec.	101	41	
5 min. 0 sec.	117	31	
7 min. 0 sec.	122	28	
10 min. 30 sec.	118	27	
11 min. 0 sec. to 11 min. 15 sec.	——	——	Injection of 4 c.c. ether solution.
11 min. 25 sec.	75	26	
12 min. 0 sec.	94	39	
12 min. 30 sec.	116	47	
14 min. 30 sec.	133	49	
15 min. 30 sec.	137	44	

Weight of dog, 12.5 kilos.

In one or two instances, the ether has failed to influence the pulmonary pressure, but in the majority of experiments in which the substance was tested, the rise in the pulmonary artery has been marked. In one instance, the pressure in the pulmonary artery rose from twenty-six to forty-seven millimeters (eighty per cent.) with a simultaneous fall in the carotid pressure of about twenty-five per cent. The second injection, ten minutes later, after the circulation had returned to normal, gave a similar rise in the pulmonary artery, but the fall in the carotid was only temporary, and the pressure subsequently rose to a point slightly above the normal (figure 3).

Alcohol.—The effects of alcohol upon the lesser circulation are, as one would expect from its chemical relation, similar in kind to

those of ether, but very much more feeble. The rise in the pulmonary arteries which generally follows an intravenous injection of alcohol, can, I believe, be justly attributed to the simultaneous widening of the blood vessels and increased output of the heart, both of which factors tend to increase the rapidity of the blood flow through the arteries, so that there is a freer supply to the right heart (Wood and Hoyt (11)).

Nitrites.—It has been abundantly shown by numerous observers, that the nitrites produce a more or less marked rise in pulmonary circulation simultaneous with the great fall in the general blood pressure. In my experience, the rise in the pulmonary artery has not been as large as I have produced with many other agents, but the relation is especially striking because of the great lowering in the carotid pressure. I have found, as has Petitjean (10), that the highest point in the pulmonary artery is reached a few seconds before the lowest point in the carotid pressure. Petitjean studied the effects of drugs upon the pulmonary blood vessels, not only through the relations in pressure, but also by changes of the color of the lung tissue. While this method is subject to the objections that it opens the way for a large psychical error, and that definite conclusions can be reached only concerning comparatively coarse alterations, it has the advantage over studies upon the rate of flow through surviving vessels, as pointed out by Petitjean, that the question does not enter of the earlier death of the constrictor fibers and the survival of the dilator vessels of the vasomotor nerve.

It is worthy of note that with this method amyl nitrite produced the greatest pallor in the lungs of any of the drugs with which Petitjean experimented. The extreme pallor of the lung, which he observed in these experiments, however, cannot be taken as evidence of violent vascular constriction, because it is probable that the increased capacity of the general vascular system diminishes the amount of blood which reaches the right side of the heart. It is all the more remarkable, however, in the face of this diminution in the blood supply, that there should be a rise in the arterial tension of the lungs.

Quebrachin.—The extraordinary influence of aspidosperma quebracho-blanco upon the respiration was first described by Penzoldt

(12). These changes have been somewhat more closely studied by Dr. D. M. Hoyt and me (13). Thinking that there was perhaps some alteration in the circulation in the lungs which might account for the extraordinary increase in respiratory activity which is seen under this drug, I have made some experiments with the effects of quebrachin, the most important alkaloid of this plant, upon the pulmonary circulation. This alkaloid in moderate doses causes in the carotid artery a transient fall of pressure, followed by a rise to normal, or sometimes slightly above. After larger doses the fall of pressure is more permanent. In the pulmonary vessels there is a slight rise in pressure irrespective of whether the general pressure falls or rises, after the first dose of quebrachin. Subsequent injections, however, even after the pressure has returned to the normal height, will not produce another rise, and if the injection is persisted in, there subsequently occurs a fall in both the carotid and pulmonary pressures.

TABLE V.

Time.	Carotid pressure.	Pulmonary pressure.	Remarks.
Beginning	160	19	Injection of 0.005 gram quebrachin.
0 min. 10 sec.	162	20	
0 min. 30 sec.	153	21	
1 min. 0 sec.	164	21	
2 min. 0 sec.	165	21	Injection of 0.01 gram quebrachin.
2 min. 10 sec.	165	23	
2 min. 30 sec.	120	22	
3 min. 0 sec.	125	23	
4 min. 0 sec.	140	21	Injection of 0.05 gram quebrachin.
4 min. 10 sec.	130	23	
5 min. 0 sec.	88	19	
7 min. 0 sec.	84	16	

Weight of dog, 13.7 kilos.

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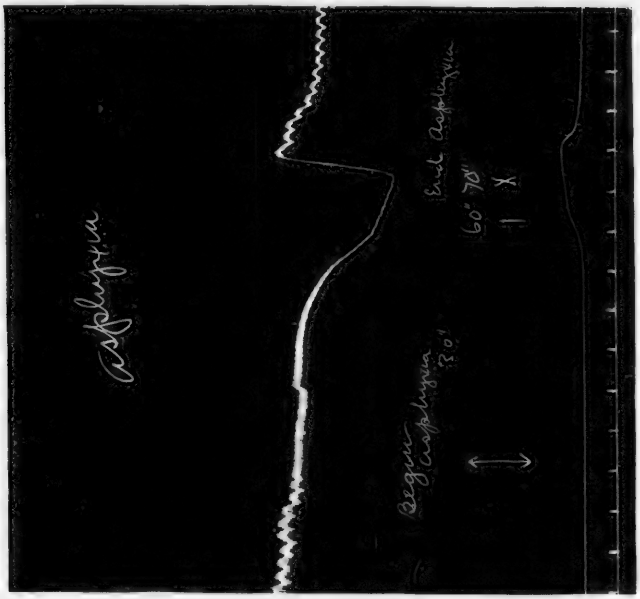

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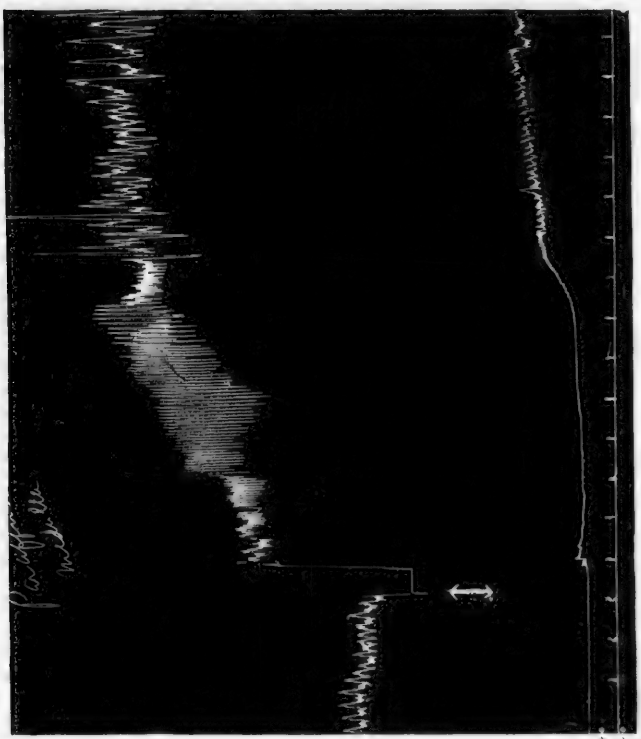
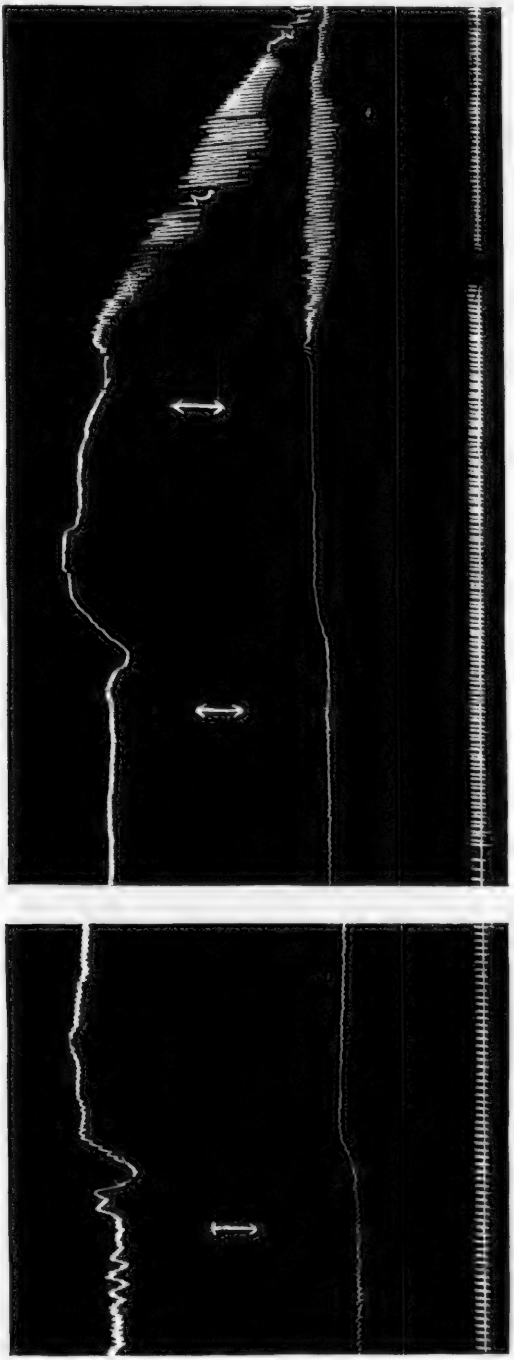


A

FIG. 1.



B

A

FIG. 2.

New

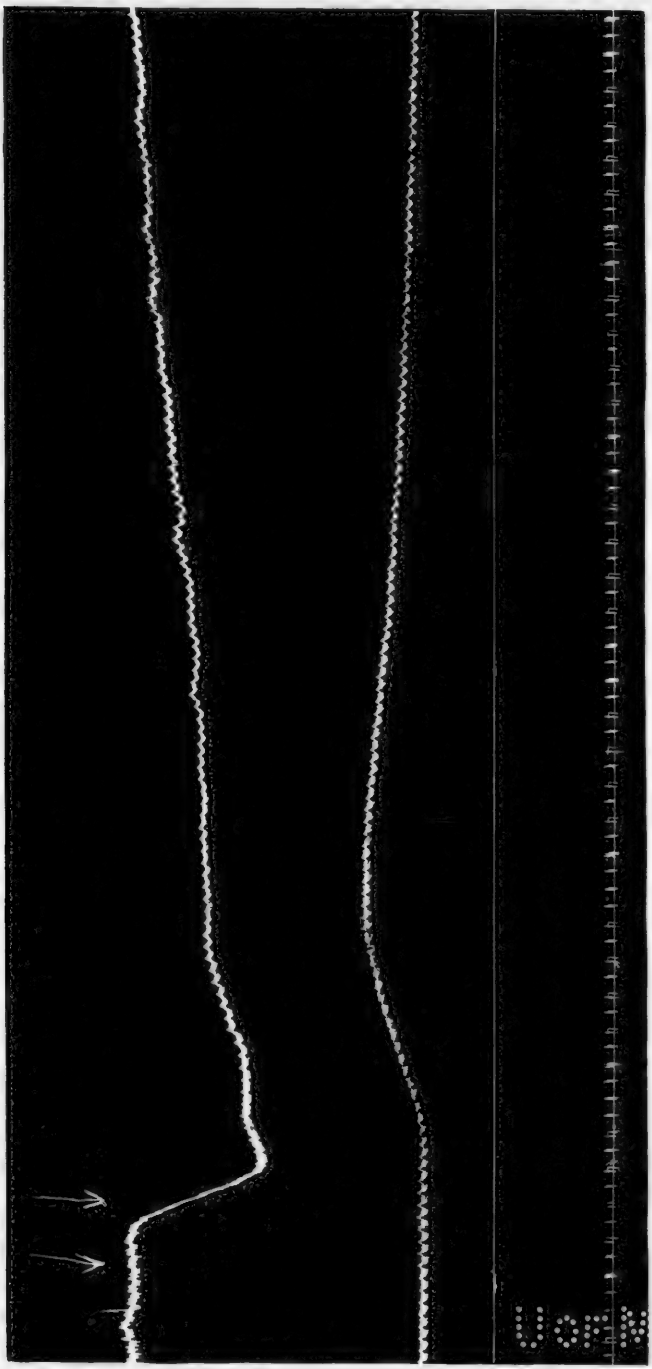


FIG. 3.

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EXPLANATION OF PLATES.

Plate 30.

Fig. 1. Time marks = 10 seconds.
(*A*) At the arrow paraffin was injected into the carotid artery.
(*B*) Six minutes later, showing the effect of 70 seconds of asphyxia.

Plate 31.

Fig. 2. (*A*) At the arrow 1 c.c. of fluid extract of ergot was injected. Time marks = 2 seconds.

(*B*) Eight minutes later. 1 c.c. of ergot has been given in the interval. At the first arrow, 1 c.c. of ergot was injected, and at the second 2 c.c. Time marks = 1 second.

Plate 32.

Fig. 3. Upper line = carotid artery; second line = pulmonary artery. Time marks = 5 seconds. Between the two arrows 4 c.c. of 33 per cent. ether solution was injected.